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ON *DINOBOTHRIUM SEPTARIA* VAN BENEDEN 1889, AND *PARABOTHRIUM BULBIFERUM* NYBELIN 1922

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ON *DINOBOTHRIUM SEPTARIA* VAN BENEDEN 1889

Dinobothrium is a genus remarkable among Tetraphyllidea and indeed among Cestoda in general for the enormous size of scolex * in its species, *D. septaria* van Ben. (7 mm. long and 9 mm. broad, according to Scott 1908) and *D. planum* Linton (8 mm. long and 10 mm. broad, according to Linton 1922). The first-named species was first very briefly and inadequately described by van Beneden in 1889 from immature material obtained from the intestine of *Lamna cornubica,* and though it has since been refound on several occasions in other specimens of the type host, in *Selache maxima* and (under the name of *D. plicitum* Linton) in *Carcharodon carcharias* (all three sharks belonging to the same family, the Lamnidae), yet, as Linton remarks, no one has yet described the structure of the ripe proglottids (remarkable in several respects) nor given an accurate and complete account of the structure of even the mature proglottids. This being the case I am glad to be able to supply a description of the anatomy of the ripe proglottids obtained from two fully-mature worms found in the hind spiral valve intestine of a small specimen (about one meter long) of *Lamna cornubica* landed at Plymouth in October (1925). This account, besides being required to fill up a gap in our knowledge, is more especially necessary since Mola in 1906 altogether misdescribed the vitellaria in mature segments of *D. septaria* (apparently mistaking the ventral layer of the ovary for the organs in question) and more recently Linton, if one assumes that the anatomy of *D. planum* is analogous to that of *D. septaria,* has repeated the mistake, since he says that "the vitellaria are distributed along the ventral side of the proglottis next within the longitudinal muscle layer" and that their lateral distribution (as seen in transverse sections of the

* So far as I am aware the scolex of *D. planum* is the largest known among Cestoda, the simple cylindriform scolices of such forms as *Priapocephalus grandis* Nybelin and *Parabothrium bulbiferum* Nybelin (see later in this paper), though several millimetres longer, being much narrower.

proglottis) is about the same as that of testes. These two descriptions imply that the arrangement of the vitellaria in Dinobothrium is different from the normal marginal dorso-ventral arrangement found in other genera of the Phyllobothriidae, which is certainly not the case in my specimens of *D. septaria,* and any argument based on the assumption that Mola and Linton are correct in this particular is no longer tenable (*vide* Baylis 1926: 171). I may also point out that the general statement made in some well-known monographs on this group of Cestodes that the vitellaria in the Phyllobothriidae and Onchobothriidae lie in the cortical parenchyma, is not strictly accurate, since in all the examples of these two families examined by me, a cortical parenchyma is not distinguishable from a medullary, the longitudinal musculature consisting of a band of scattered bundles lying in and immediately under the subcuticula, and the vitellaria, with the testes and other organs, lying internal to this. The vitellaria, thus placed, may be described as cortical, but so also may the ovary and other organs.

Van Beneden (1889) very briefly described the external anatomy of the single immature worm found by him, the length of which he gives as 25 to 30 mm., though his figure would seem to indicate a worm larger in size, i. e. 125 to 130 mm. in view of the elongated terminal proglottids. He describes "une petite ventouse" as lying above each bothridium, "dont on ne voit que la moitié de la circonférence, et qui est collée à une portion saillante, comme le nid de l'hirondelle Salangane est collé au rocher," and every subsequent author appears to have accepted van Beneden's word for this protuberance being a sucker. It is also important to note that van Beneden's figure shows some indication of the "furrow at the middle of the posterior border" (Linton) of the bothridium described by Linton in the case of *D. "plicitum."*

In 1892 Lönnberg found immature *D. septaria* again in *Lamna cornubica* and described the scolex in some detail, and again in 1898 he obtained a number of young specimens from the same host (renamed *Isurus cornubicus*), the longest of which attained a length of 18 cm.(!) Even in this 180 mm. worm the terminal proglottids were apparently still devoid of eggs. He described the genitalia of the mature proglottid (more than twice as broad as long) and correctly figured the curious anterior course of the vagina but could not describe the vitellaria which, in his material, were either not developed or only slightly developed. In 1906 Mola obtained material from *Selache maxima* from the Mediterranean, the strobila measuring 80 to 120 mm. in length. He described the anatomy of the mature proglottid only, and made several mistakes in connection with the course of the vagina, the shape of the ovary and the distribution of the vitellaria. Scott in 1908 obtained a specimen from *Lamna cornubica* from the North sea which measured about 45 mm. in length, Masi in 1912 another specimen from *Selache maxima* from the

Mediterranean and Nybelin in 1914 again recorded *D. septaria* from *Selache maxima* caught off Sweden, and provided photographs of the scolex. Finally Linton in 1922 has published an account of the scolex of *D. "plicitum,"* a species obtained from *Carcharodon carcharias* and which I believe to be a synonym of *D. septaria,* and of the entire anatomy of fully mature specimens of *D. planum,* from *Cetorhinus maximus,* which is undoubtedly a new species, differing in many respects from *D. septaria,* as will be seen.

SOME EXTERNAL CHARACTERS OF *Dinobothrium septaria*

One of my two specimens of this species measured 110 mm. in total length and about 2.5 mm. in maximum breadth, and the other (unmeasured) was of about the same dimensions, and both bore fully-ripe terminal segments. This last fact is surprising in view of Lönnberg's statement that even in one of his specimens measuring 180 mm. the terminal segments were not yet ripe, and of Nybelin's similar statement that no mature segments were to be found in worms measuring 130 to 155 mm. In the case of each worm I fixed the scolex and a portion of the anterior strobila in hot 6% formalin (afterwards transferred to alcohol) and the remainder of the strobila I cut into portions, some of which I flattened between glass slides in 6% formalin or 70% alcohol (for whole mounts) and others I fixed unflattened (for sections) in hot formalin. In most cases the portions of strobila were ultimately hardened in alcohol and stained either with very dilute borax carmine (an excellent method for whole mounts), or with Delafield's hematoxylin or very dilute Mayer's acid hemalum combined with eosin. One of the scolices was sectioned sagitally; the other was left intact and unstained in spirit. It is very essential in the case of all Cestodes to retain some ripe proglottids in formalin for the examination of the eggs, since these become distorted when transferred to alcohol.

Linton's description (1922) of the scolex of his new species *D. plicitum* corresponds well on the whole with the characters of the scolex of the present species, and in the absence of contrary evidence to be derived from an examination of mature and ripe proglottids of Linton's species, I can only conclude that his material consisted of immature examples of *D. septaria* and therefore does not represent a new species. The "striking feature" of the bothridium of *D. "plicitum,"* viz. the "furrow at the middle of the posterior border," is also to be found well developed in my examples of *D. septaria,* though it has not always been figured in this species by previous authors. In Linton's examples of *D. "plicitum"* the bothridia had a maximum length of 4 mm. and maximum breadth of 2.5 mm. (the immature strobilae having in alcohol a maximum length of only 20 mm.).

In Scott's (1908) material (with an immature strobila about 45 mm. long) the bothridium was about 7 mm. long and about 4.5 mm. broad. In Nybelin's material (from *Selache maxima* and with immature strobilae measuring 130 to 155 mm. long!) also the bothridia of two scolices measured 7 mm. long and about 4.5 mm. broad. In my fully mature material the bothridium of my intact scolex measured (in alcohol) 5 mm. in length and 3.3 mm. in breadth and the anterior end of the scolex (i. e. the straight parallel crests lying above the two bothridia of each side) 5 mm. in length.*

Most authors describe the presence of four small suckers on the anterior crests of the large bothridia and these bodies are represented in my material by four small slightly dimpled swellings in the position indicated in figure 1. But these swellings, in my material, are not suckers, as can be demonstrated by serial sagittal sections. They merely consist of enlarged protuberant areas of the crests which lie above the bothridia and, like the remainder of the crests and the walls of the bothridia themselves, contain muscle fibres lying roughly at right angles to the surface (Figs. 2, 3). It is true that the muscle fibres of these four swellings show a slight disposition to be separated from the other fibres of the crest but not to a greater extent than in other local areas, and the fibres of the whole of the crest are more or less segregated from the fibres of the walls of the bothridia save at places near the outer edges of the bothridia where the fibres of the two structures (the bothridial walls and the crest) are more or less continuous. Definite sucker-like organs, possessing a lumen and distinguishable from all surrounding structures, are entirely absent. Since van Beneden, Linton, Lönnberg (1892) and others have already described and figured the scolex of *D. septaria* in detail and good figures are given (after van Beneden) by Braun in Bronn's Thier-reich, further re-description of the scolex is unnecessary. I only wish to emphasize the facts that the furrows on the posterior borders of the bothridia of *D. "plicitum"* described by Linton are also present in my specimens of *D. septaria,* that the size of the scolex for a given length of strobila varies considerably and that the four suckers so often described as being present on the crest above the bothridia are not functional suckers in the accepted sense of the term but only localized swellings apparently of no particular significance.

An unsegmented neck is absent, the narrow anterior region of the strobila being distinctly segmented up to its junction with the scolex (Fig. 1). All the proglottids, save the terminal ripe proglottids, are broader than long and all are distinctly flat in transverse section (Fig. 14). The proglottids are delimited, one from the other, by

* Measurements which should probably be multiplied by about 3/2 to represent the dimensions of the living worm.

slightly salient edges, by small marginal notches and by distinct lines of segmentation. Ripe proglottids, i. e., proglottids with a well-expanded uterus filled with eggs and with a distinct very large opening from the uterus to the exterior, are relatively few in number (less than a dozen in each of my two worms), are distinctly longer than broad (3.5 mm. long by 1.5 mm. broad and 3 mm. long by 2 mm. broad in only slightly flattened balsam-mounted specimens) and with anterior extremities which appear in each case to form a distinct kind of articulating joint (enclosing a hollow space or "bay") with the hind surface of the preceding proglottid (Fig. 13). Since most of these ripe proglottids in my two worms have already largely discharged their eggs, it is almost certain that they do not become detached and lead an independent existence, as is the case in many other Tetraphyllidea. The genital apertures are marginal, irregularly alternate and always situated a short distance behind the middle transverse line of the proglottid.

THE MALE GENITALIA OF THE RIPE PROGLOTTID

The testes lie for the most part in two lateral fields, situated, in ripe proglottids, between the walls of the vagina and uterus in the center, and the excretory vessels marginally, and somewhat dorsally and extending posteriorly as far as the ovary. In transverse sections of ripe proglottids (Fig. 7) they are seen to lie as a whole more centrally than the vitellaria which latter lie immediately under the longitudinal muscle bundles and occupy the outer third of the body on each side, extending external to the excretory vessels. In these transverse sections the testes are spherical in shape and measure on the average about 51 by 47μ. The thickness of the longitudinal muscle layer in ripe proglottids renders it difficult to observe the testes in whole mounts and I did not cut horizontal sections.

The cirrus sac is a large elongated body, with a thin though distinct muscular contractile wall, which opens on the margin of the proglottid at a point always situated a little behind the middle transverse line of the segment. From this point the sac extends inwards and bends forwards, its inner part reaching the middle longitudinal axis of the segment (Fig. 6). The sac, containing the coiled-up cirrus, measures in different balsam-mounted proglottids 0.99 to 1.32 mm. in length and 0.38 to 0.44 mm. in breadth, but when the cirrus is extruded in whole or in part the sac contracts greatly. The cirrus sac and vagina both open on the same dorso-ventral level into the bottom of the large genital atrium, the former posteriorly, the latter anteriorly (Fig. 10). The atrium itself usually opens on the extremity of a prominent papilla (Fig. 6) but this is not always visible (Fig. 4). The cirrus consists of two parts, both eversible. The proximal part (i. e. next to the cirrus sac opening) is thick-walled, usually of large diameter and usually

nearly or quite as long as the cirrus sac itself, and this proximal part tapers into the distal part, the diameter of which is at least one third of that of the proximal part, though its length is only about one and a half time as great. Both parts, in the introverted condition, are lined with spines which, on eversion, cover the outside of the long cirrus. The spines of the proximal part (about 18μ long) are stouter than those of the distal part (about 11μ long). I have several examples of the proximal thick portion of the cirrus being more or less extruded to the exterior through the atrial cavity but none in which the distal thin portion is also thus everted. On the other hand, in four cases the cirrus is seen to be everted direct into the vagina of the same proglottid instead of to the exterior and in one case the whole of the cirrus (proximal and distal portions) is thus everted and extends a considerable distance up the vagina convolutions (Fig. 4), the cirrus sac having shrunk considerably. The vas deferens, as it emerges from the cirrus sac, is thin, but, while in the vicinity of the sac and with the cirrus unextruded, it almost immediately dilates to form a much-coiled sperm reservoir packed with spermatozoa, and only subsequently again becomes thin, the convolutions extending forwards and inwards to the middle of the proglottis and thence to the testes.

THE FEMALE GENITALIA OF THE RIPE PROGLOTTID

The vagina exhibits a peculiarity which though of minor significance is yet very striking and distinguishes it from at least the majority of other Cestodes. The first thick-walled portion of the vagina is often almost as wide as the cirrus sac and of about the same length, is straight and lies next or close to and parallel with the sac, i. e. it extends from its opening directly forwards and inwards. The subsequent portions of the vagina become successively more narrow, are thin-walled and exceedingly convoluted and extend in the first instance to the extreme anterior border of the proglottis in the middle line, whence the convolutions turn back at a sharp angle and, continuing in the middle longitudinal axis of the proglottid, become progressively narrower and extend back to the middle region of the ovary (Figs. 4, 6). This remarkable forward extension of the vagina, previously described by Lönnberg in the mature proglottid but apparently not observed by Mola, implies an enormous capacity for storing spermatozoa and is probably correlated with a brief but energetic period of sperm production. It is at least certain that the voluminous vagina leaves but little space for the presence of other organs and, as is shown later, the eggs are very quickly liberated from the uterus by the extensive splitting of the ventral body-wall and are not stored in any considerable quantity. The vagina opens posteriorly into the oviduct just behind the egg-ejector or "Schluckapparat," and close to this opening the vitelline ducts enter, and the oviduct,

after receiving the contents of the shell-gland, continues as the uterine duct. The uterine duct (Fig. 14) is narrow at its origin and throughout its length and runs forward, on the dorsal side of the vagina, as far, in mature short segments, as the middle of the length of the cirrus sac where it bends ventrally and opens into the uterine sac, but in ripe elongated segments it opens into the uterus only a short distance in front of the ovary (Figs. 11, 12). In ripe segments (Figs. 8 to 11) the uterus proper or uterine sac, as one may call it to distinguish it from the narrow uterine duct, becomes greatly dilated (though constricted between the coils of the enormous vagina), develops saccular outgrowths at its sides (except in the vicinity of the cirrus sac) and extends back even to the hind border of the ovary, the saccular outgrowths lying between the dorsal and ventral layers of this organ (to be described). Owing to the enormous development of the vagina the uterus, despite its development, has but little space in which to store eggs and in all ripe segments the ventral wall of the proglottid develops an enormous gape through which the eggs escape from the ruptured wall of the exposed uterus (Figs. 6, 8 to 10). This gape or secondary uterine aperture is a conspicuous feature in all the elongated ripe proglottids and it contrasts vividly with the normal neat circular uterine pores found in most Proteocephalids and Bothriocephalids. It is apparently very similar to the elongated gape formed in Beddard's *"Solenotaenia" viperis* (*vide* Woodland 1925b), though in this latter the whole uterus is very small and undeveloped, whereas in *D. septaria* the uterus sac and its outgrowths are fairly extensive—a difference probably due to the late development of the gape in the latter species. The eggs contained in the uterus of a ripe proglottid preserved in formalin are spherical, contain spherical embryos and possess a distinct shell, with a diameter of about 25.6μ (in formalin). The embryos had not developed hooks in my specimens.

The ovary, in ripe segments (Fig. 12) consists on each side of the body of two thin (especially so in merely mature segments: *vide* Fig. 14) sheets of tissue, a dorsal and a ventral, each lying immediately under the layer of longitudinal muscles and separated anteriorly by the divisions of the uterus, except in the median line where they converge and join on each side of the median vagina. Except in the most anterior region of the ovary, the two halves of the ovary unite across the middle line under the vagina. Towards the hind end of the vagina, when this has become smaller, the junction of the two halves of the ovary forms the isthmus or ovarian reservoir, which lies ventral to the vagina, and behind the isthmus the vagina narrows and turns ventrally to open into the oviduct behind the "Schluckapparat," as already described. The ovary, seen in transverse section, thus has the

form of an X with the arms pulled out horizontally. This form of ovary is probably characteristic of all Phyllobothriidae and Onchobothriidae and constitutes a good character for the definitions of these families as apart from the Proteocephalidae and probably all other Cestodes except Tetrarhynchidae. In surface view of whole mounts of ripe proglottids and in sections the ovary can be seen to lie at the extreme posterior end of the proglottid and does not occupy more than one sixth of the proglottid length, and transversely it extends from the middle line to the zone occupied by the marginal vitellaria.

The vitellaria, seen in transverse sections of a ripe proglottid (Figs. 7 to 13), consist of a row of vesicles situated immediately under the longitudinal muscle layer in the marginal zone on each side of the proglottid. This row extends externally to the two excretory vessels and nerve of each side and so forms a loop, testes, in the region of these organs, separating the dorsal and ventral arms of the loop on the inner side of the vessels. In the region of the ovary the arms of the vitellarian loops become almost contiguous with the sheets of ovary tissue. The vitellaria thus extend along the entire length of the proglottid and are quite typically Tetraphyllidean in arrangement. In these transverse sections the vitellaria attain a maximum size of about 40 by 26μ.

From this account (and the arrangement is the same in my sections of merely mature proglottids, as shown in Fig. 14) it will be seen that my preparations afford no evidence in support of Mola's supposition that the vitellaria lie in a sheet ventral to a small branching ovary and nowhere else. This author has evidently mistaken the ventral part of the laterally-extended ovary for the organs in question, and it must be admitted that in some sections the two closely resemble each other histologically, though otherwise they are quite distinct. Nor do my preparations support the similar statement of Linton regarding the vitellaria of *D. planum,* and since there is not the slightest doubt concerning the truth of my own description (which has been confirmed by Dr. Baylis), and *D. planum* is not likely to differ anatomically to such an extent from *D. septaria,* I feel pretty confident that Linton has erred. Baylis (1926), on account of certain strong resemblances between the scolices of *Dinobothrium septaria* and *Tetrabothrius affinis,* suggested that these two species "must be very closely related" and that "*Dinobothrium* ought perhaps to be regarded as a member of the family Tetrabothriidae" rather than as a Phyllobothriid, and he cited Linton's statement (regarding the supposed Tetrabothriid-like restriction of the vitellaria to the ventral surface) in support of his suggestion. Curiously enough, since writing the above description of *D. septaria,* I have examined the structure of another primitive Cestode which at first I imagined was allied both to the *Priapocephalus grandis* of Nybelin (since it possesses an almost identical scolex) and to the *Diplobothrium simile*

(also from *Lamna cornubica*) of van Beneden, and in this Cestode, the *Parabothrium bulbiferum* of Nybelin (see later), the vitellaria do present the arrangement erroneously supposed by Mola and Linton to exist in *Dinobothrium*, but *Parabothrium* is a Pseudophyllidean and there is good reason to think that Nybelin (1922) is right in suggesting that the Tetrabothriidae have Pseudophyllidean affinities rather than Tetraphyllidean, despite the vestigial condition of the dorsal uterine openings in this group. I may also add that *D. septaria* is a typical Phyllobothriid and, apart from certain superficial features of its scolex, shows no affinity with Tetrabothriidae.

THE MUSCULATURE AND EXCRETORY AND NERVOUS SYSTEMS

In *Dinobothrium septaria* the subcuticula and longitudinal muscle layer are constituted as in other Phyllobothriidae and Onchobothriidae. In the present species the cuticle is thin (about 2.7μ) though dense; on the other hand, the nuclear subcuticular layer underlying the cuticula is very deep and in it and extending well below it are the numerous fibres and bundles of fibres of the longitudinal muscle layer (Fig. 5). The muscles immediately underlying the cuticula either consist of separate fibres or of small bundles of fibres, and, in these bundles the constituent fibres are arranged in rows at right angles to the cuticular surface. The more deeply situated the bundles the larger they become and the largest bundles (consisting of from 10 to 15 fibres) are situated internal to the subcuticula. There is no means of distinguishing an inner from an outer layer of longitudinal muscle bundles. Between the muscle bundles the nuclei of the subcuticula are visible. Below the layer of muscle bundles the parenchyma is traversed by occasional dorso-ventral fibres (numerous where the parenchyma is not occupied by organs) but, save for a very thin circular layer of fibres immediately underlying the cuticula, I have observed no other kinds of fibres. In this Cestode therefore, as in other Phyllobothriidae and Onchobothriidae which I have examined, there is no distinction between cortex and medulla and therefore there is no foundation for the common text-book statement that the vitellaria are cortical in these two families, a statement which I unfortunately repeated in a recent paper and represented in a diagram.

At the extreme anterior end of the ripe proglottid the relatively large ventral and the small dorsal excretory vessel on each side are both rather small but these soon increase in size and maintain a fairly constant diameter as they proceed posteriorly. At the extreme posterior border of the segment the small dorsal and large ventral vessel of each side turn inwards towards the middle line of the segment and open independently into the bay or inlet which is formed at the hind end of each proglottid, i. e. to the exterior (Fig. 13). The difference in size therefore of the dorsal and ventral excretory vessels has therefore

apparently nothing to do in this case with ascending or descending currents; the two kinds of vessel probably drain separate sets of tissues. On the other hand, in merely mature or immature segments the vessels appear, in my serial sections, to be continuous from segment to segment and to have no intersegmental openings. The two vessels lie close together save where they become separated dorso-ventrally (the lateral nerve retreating dorsally with the dorsal vessel, only the ventral vessel remaining ventral to the sac) to allow the cirrus sac and vagina to reach the exterior. The lateral nerve lies just external to the two excretory vessels of each side and is quite conspicuous.

THE SPECIES OF DINOBOTHRIUM AND VALIDITY OF THE GENUS

In the absence of information concerning the characters of the mature and ripe proglottids of Linton's *D. plicitum,* I can only assume, with Southwell (1925), that this species, obtained from *Carcharodon carcharias,* a shark belonging to the same family as *Lamna cornubica,* is indistinguishable from *D. septaria.* On the other hand, Southwell is certainly wrong in assuming that Linton's *D. planum,* from *Cetorhinus maximus* belonging to the family Cetorhinidae, is also indistinguishable from *D. septaria.* On the contrary, *D. planum* constitutes a very distinct species, differing from *D. septaria* in the following obvious features: (1) the strobila of *D. planum* is much larger than that of *D. septaria,* known specimens attaining a maximum length of 825 mm. and a breadth of 4 mm. and more; (2) the ripe proglottids of *D. planum* are always much broader than long (one tenth to one sixth as long as broad) whereas in *D. septaria* fully-ripe proglottids are always longer than broad (one and a half to twice as long as broad, and half-ripe proglottids are at least not much broader than long, judging from my flattened preparations); (3) the genital apertures of *D. planum* are situated about the middle of the proglottid length or a little anterior and never posterior as is always the case in *D. septaria;* (4) the cirrus pouch and external portion of the vagina in *D. planum* are relatively small and lie at right angles to the proglottid margin and not large and inclined forwards as in *D. septaria;* (5) the vagina of *D. planum* runs direct to the middle line and then backwards, whereas in *D. septaria* it first runs forward to the extreme anterior border of the proglottid in the manner described; (6) the conspicuous splitting of the ventral body-wall in *D. septaria* to allow of the escape of eggs from the uterus is apparently absent in *D. planum.*

Southwell contends that the genus Dinobothrium cannot be maintained as distinct from Phyllobothrium—"since each bothridium bears an accessory sucker the genus is indistinguishable from Phyllobothrium (van Ben.)." I have no intention here of entering upon a discussion as to the validity of Phyllobothriid genera and I will content myself with

remarking that it appears to me, in view of the very distinct and constant form of the large one-loculed crested bothridium of the scolex in the two species of *Dinobothrium,* that this character constitutes, in the absence of more suitable features, a more reliable basis for the formation of a genus than the presence or absence of accessory suckers, structures very variable in occurrence, often "difficult to see," and I may add, often supposed to be present when they are not, as in the present case of *D. septaria.* The only alternative, in the present state of our ignorance of the anatomy of these forms, to the founding of numerous genera solely based on the more conspicuous varieties of bothridium found in the Phyllobothriidae is the inclusion of all the species in one genus Phyllobothrium, but until this procedure is adopted I think the genus Dinobothrium must be considered as well founded as any of the other genera listed by Southwell or other authors.

ON *PARABOTHRIUM BULBIFERUM* NYBELIN 1922

Though Nybelin (1922) has given a fairly complete account of the anatomy of the genital organs of this species yet he has omitted to describe the structure of the "scolex deformatus," the excretory system and some other organs. I think it well therefore to supplement his description with a redescription based on some fresh material which I obtained at Plymouth in 1925, especially since I agree with him that the anatomy of this Pseudophyllidean species may throw some light upon the question as to the affinities of the aberrant family of the Tetrabothriidae, a family usually placed with the Cyclophyllidea.

My material consisted of three worms or portions of worms collected from three Pollacks (*Gadus pollachius* L.). In one fish I found one immature worm about 70 mm. long, the strobila of which lay in the anterior intestine but its scolex had penetrated through the gut wall and was therefore in the coelom. In a second fish I found a much larger incomplete specimen which measured about 260 mm. in length but it was apparently devoid of the anterior portion of the scolex, only the base remaining attached. In a third fish I found on the mesentery, i. e. in the coelom and not the gut, a small immature fragment (about 12 mm. long) of the strobila of another worm, and no other remains. The scolex of the 70 mm. worm I cut off and fixed in hot 6% formalin, and the immature strobila I flattened between glass slides in cold formalin for toto-mounts (stained with very dilute borax carmine). The scolex I first drew as a whole and subsequently cut into sagittal sections, after removing the loose cuticula. The largest worm I fixed in the same way and cut into portions, some flattened for toto-mounts, others unflattened for sections. It is possible and even probable that the protrusion of the scolex through the gut wall in one fish and the fragment of strobila being found in the mesentery were both due to the fish having been dead some

hours before I examined them, though it is curious that I found no other remains of the worm in the third Pollack. Van Beneden (1871) notes that the head of his *"Abothrium gadi"* may pass into the abdominal cavity and Baylis (1926) states that in the specimens of *Priapocephalus grandis* (with a scolex almost identical in form with that of the present species) examined by him "the entire scolices (including the "collar") were buried deeply in the mucous membrane of the intestinal wall" of the host.

I possess one scolex only: that of the 70 mm. worm. In this worm the scolex measured, when preserved in formalin, about 13 mm. in length (from the apex to the base of the "collar") and about 3 mm. in maximum breadth, and was of the simple tapering cylindrical form shown in Figures 15 and 16. It was covered with a very thick cuticula which, in the formalin and perhaps in life had become separated from the underlying subcuticula except at the apex and the base. On removal of this cuticula the substance of the scolex presented the appearance shown in Figure 17 and the "collar" was seen to be non-existent apart from the cuticula. In serial sagittal sections (Fig. 18) the circum-medullary band of longitudinal muscle bundles found in the strobila is seen to disperse on entering the scolex, individual fibres penetrating the whole parenchymal mass up to the apex. The only other structures observed in the substance of the scolex were the extensions of the two longitudinal nerves, which end at the extreme apex and may have connection with a patch of what appear to be ganglion cells, and of the excretory vessels. No traces of suckers or phyllidea were found. The scolex therefore closely resembles that of *Priapocephalus grandis,* as figured by Nybelin (1922) and Baylis (1926).

The maximum breadth (in alcohol) of the strobila of the 260 mm. worm was about 5 mm. and in this region the individual proglottids in one of my preparations are more than 11 times broader than long. In more anterior regions measuring only 2.7 mm. broad however, the proglottids are 0.354 mm. long, i. e. only a little more than 7 times as broad as long, but these variations, perhaps due to degree of contraction, are but of little importance. The proglottids are demarcated by distinct grooves and lateral notches but, especially in the broader region of the strobila, there are also often one or two secondary grooves and two or three secondary notches (including those at the genital pores), almost as well marked, intervening between the primary grooves and notches. The strobila is also marked by seven (or eight) longitudinal grooves (Fig. 20) which extend along the greater part of its length. An unsegmented neck is absent. The marginal genital pores lie in the anterior third of each proglottid, and, as I have already stated, are irregularly alternate (Fig. 19). The narrow vagina is constantly on the anterior side of the cirrus pouch and ventral to it.

Taking the layers of body-substance (apart from the genital organs) in mature proglottids in order from the exterior inwards (Fig. 24), the (1) cuticula is of very uniform thickness (about 15μ in transverse sections) and immediately underlying it (2) a thin layer of circular muscle fibres, (3) and a thin layer of fine longitudinal muscle fibres, the cuticular longitudinal muscle layer, hitherto undescribed. Next comes (4) the nuclear layer of the subcuticula which is from two to three times as thick as the cuticula and bounds externally (5) the cortical zone of parenchyma which, apart from the area occupied by the longitudinal muscle band, is about twice the thickness of the nuclear layer and contains dorso-ventral muscle fibres and calcareous bodies. Bounding the cortex internally is (5) the single layer of longitudinal muscle bundles, which are large, variable in shape and size and consist of numerous fibres, and internal to these is (6) a very distinct though thin layer of transverse muscle fibres which mark the outer limit of (7) the medullary parenchymal zone, in which lie all the genital organs, the excretory channels and the longitudinal nerve trunks. The medullary zone occupies less than one quarter of the dorso-ventral diameter of the proglottid.

The excretory system is of an unusual type, since transverse sections across mature proglottids show on the average no fewer than some twenty longitudinal canals running in the substance of the medulla (Fig. 21). In serial sections across one entire proglottid the number of vessels counted in successive sections varied between 18 and 23, about half being small thick-walled dorsal vessels and the other half relatively large thin-walled ventral vessels, but some of the vessels seen represented anastomoses between the longitudinal canals. These canals are continued into the scolex to near the anterior extremity.

The central nervous system consists, as usual, of two longitudinal nerve trunks, one on each side, lying well internal to the outermost testes and excretory canals (Fig. 20), and running dorsal to the cirrus sac when crossing it. Both nerve trunks are continued to the extreme apex of the scolex, where they appear to be connected with a patch of ganglion cells.

THE MALE GENITAL ORGANS

Most of my observations on the structure of the genital organs have been made from serial transverse sections, since in toto-mounts the internal organs are largely obscured by the thick longitudinal muscle sheath, and I may add here that in none of my preparations can the genitalia be described as more than young mature. The testes in transverse sections of mature proglottids lie in a single row in the center of the medulla, measure on an average about 55 by 62 μ, and extend over the entire area of the medullary parenchyma save where this is occupied by other organs such as the cirrus sac and vas deferens, ovary and the median uterus.

The cirrus sac is elongated (extending over from one-sixth to one-fifth and sometimes to nearly one-quarter of the proglottid breadth) and narrow (about four times the breadth of the adjacent vagina) and is very thick-walled and muscular; on the other hand, the contained "cirrus" is practically straight throughout its length and is very thin-walled save for a thick-walled bulbous expansion situated at the inner end of the "sac," next to the vas deferens. The enormous thickness of the wall of the "sac" and the thinness of the "cirrus" wall would almost seem to indicate that the whole structure is a cirrus and that a sac proper is absent. Unfortunately in none of my preparations is the cirrus extruded. The genital atrium is of simple form with slightly muscular walls, measures, in my sections, about 99 by 66μ and opens on the proglottid margin midway in the vertical depth of the proglottid and in the anterior third of the proglottid length on either side of the strobila. The vas deferens, which emerges from the inner extremity of the thick-walled "sac," is narrow and very convoluted and runs, dorsal to the vagina, to the middle line where it subdivides.

THE FEMALE GENITAL ORGANS

The vagina in my transverse sections (Fig. 23) is a narrow tube, sometimes very slightly dilated next to its external aperture, which opens into the genital atrium anterior to the cirrus sac and lies immediately anterior to the sac or somewhat below it. It runs almost directly to the center of the proglottid, lying ventral to the vas deferens and inclining posteriorly and dorsally as it reaches the center, and thence it runs posteriorly (dorsal to the uterus) with one or two small convolutions and opens into the oviduct a little anterior to the egg-ejector ("Schluckapparat"). In my young material the vagina shows practically no dilatations.

The ovary (Fig. 19) is a median flattened irregular body lying at the posterior end of the proglottid and in transverse sections practically occupies the whole vertical depth of the medulla and extends laterally over a little less than one quarter of the proglottid breadth. The egg ejector arises from the ventral side of the median part of the ovary and is a small pear-shaped body which opens anteriorly into the oviduct, which almost immediately receives the opening of the vagina and later the junction of the two ventrally-placed vitelline ducts. The region of the oviduct next anterior to this develops later a shell-gland on its wall but my material is too young to show it. The oviduct, now become the uterus, after several convolutions opens into a spherical muscular chamber with a wide lumen, situated just behind the point at which the vagina and vas deferens reach the middle line of the proglottid from their marginal openings. From this muscular chamber a downgrowth, still containing a wide lumen, extends vertically downwards and ends blindly

just beneath the cuticula in the middle line of the ventral side of the proglottid (Fig. 24). In more mature segments a median ventral uterine aperture is formed here but no external openings could be detected in my material.

The vitellaria are only to be found on the ventral side of the medulla (Fig. 22) and in the posterior half of the proglottid, i. e. from well behind the cirrus sac to near the hind end of the ovary. They lie in two lateral fields, between the vertical levels of the outermost testes and the inner limit of the outermost third of the proglottid breadth, i. e. they do not extend inward (mediad) so far as the outer edges of the ovary. They are not very numerous and are small, measuring on an average, in transverse sections, about 22μ in diameter. The vitelline ducts of the two sides are ventral and unite just before they open into the oviduct.

SCOLEX FEATURES AS GENERIC CHARACTERS

As already remarked, the external features of the scolex of *Parabothrium bulbiferum* closely resemble those of the scolex of the Tetrabothriid *Priapocephalus grandis,* as figured by Nybelin (1922) and Baylis (1926), and I was at first under the impression that my examples of the former species belonged to the family of the Tetrabothriidae, since in both species, in addition to the similar forms of scolex, the proglottids are very short in comparison with their breadth and the musculature (longitudinal and transverse), nervous systems and general disposition of the organs are of the same type, and I was disposed to think that the irregularly alternate arrangement of the genital pores, the elongated cirrus sacs and the scattered ventral vitellaria merely denoted a primitive condition, and it was not until I found the convoluted median uteri and ventral uterine pores that I found reason to doubt the correctness of my impression. This impression had been further supported by the facts that the *Diplobothrium simile* of van Beneden, which possesses a typical Tetrabothriid scolex said to be almost identical with that of Lönnberg's *Diplobothrium affine* (= *Tetrabothrius affinis*), also possesses, according to Lönnberg's incomplete account (1892), irregularly alternate genital pores, elongated cirrus sacs, scattered vitellaria and median uteri. Until we possess an accurate complete description of the anatomy of van Beneden's *Diplobothrium simile* it is impossible to know for certain whether this worm is a Phyllobothriid or a Bothriocephalid, but one thing we can be almost certain about is that it is not a Tetrabothriid, and if this be so, the significant fact remains that here is a non-Tetrabothriid worm possessing a scolex closely resembling that of the very typical Tetrabothriid *Tetrabothrius affinis* (*vide* Baylis 1926). This fact, together with the close resemblance between the "deformed" scolices of the Bothriocephalid *Parabothrium bulbiferum* and the Tetrabothriid *Priapocephalus grandis* above referred

to and the similarities between the scolices of *Tetrabothrius* and of the Phyllabothriid *Dinobothrium septaria,* pointed out by Baylis, proves once more to my mind (*vide* Woodland 1925a, p. 385) that the scolex is a very unsafe guide in the detailed classification of Cestoda. It is true that Cestodes with scolices possessing two bothrial grooves, four "proboscides" and four phyllidea (without proboscides) are certainly Bothriocephalids, Tetrarhynchids and Tetraphyllidea respectively but to assert that all Cestodes with scolices possessing four "true" suckers must *ipso facto* belong to the Cyclophyllidea and that all Cestodes with scolices devoid of "four suckers, four bothridia, four proboscides or two bothria" can even provisionally be grouped together in a separate order, the Heterophyllidea (Southwell 1925), is to ignore some very important facts. These facts are that atypical scolices are to be found in all the orders and that scolices with suckers (often four in number) are to be found in orders other than the Cyclophyllidea (since phyllidea and perhaps even bothrial grooves can assume the forms of suckers, and so-called accessory suckers are of frequent occurrence), that knowledge of the anatomy of most of these forms with atypical scolices is extremely incomplete, and that a constant form of ovary or uterus, the presence or absence and the position of a uterine aperture, the disposition of the vitellaria, and the dorsal or ventral position, relative to the ovary, of the posterior genital ducts, are characters of much more vital importance, though doubtless of far less convenience, for classification than the idiosyncrasies of form of an external organ of attachment.

In conclusion I wish to acknowledge my indebtedness to Dr. E. J. Allen, F.R.S., and other members of the staff at the Plymouth Marine Biological Laboratory for much assistance in the collection of Cestode parasites from fishes during my two months stay at Plymouth in 1925, to Professor J. H. Ashworth, F.R.S., and other members of the Plymouth Station Committee of the British Association for kind permission to occupy the Association Table, to Dr. H. A. Baylis for some criticisms and to Miss I. M. Bellis for assistance in translation.

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Explanation of Plates

Abbreviations used

ATR genital atrium (sinus genitalis)
BO bothridium
C cortex
CC cut edge of cuticula
CIR cirrus
CIRM thin circular muscle layer under cuticula
CR crest of bothridium
CS cirrus sac
CUT cuticula
DEC dorsal excretory canal
DVM dorso-ventral muscles
EE egg-ejector ("Schluckapparat")
EXC excretory vessel
LM longitudinal muscles
M medulla
N nerve
O ovary
OD oviduct
S so-called "sucker"
SP "bay" from the exterior at the hind end of the proglottis
SUB subcuticular
SUBLM thin subcuticular longitudinal muscle layer
TES testes
TRM transverse (circular) muscle layer
U uterus
UD uterine duct
UTD ventral uterine downgrowth towards exterior
UTO uterine aperture
VAG vagina
VAG PROX proximal inner descending portion of vagina
VD vas deferans
VEC ventral excretory canal
VIT vitellaria

Explanation of Plate XII

Dinobothrium septaria van Ben.

All figures drawn under the camera lucida

Fig. 1.—Scolex viewed end-on and partly edgewise, to show the four so-called "suckers" (*S*) and the four crests (*CR*) of the bothridia (*BO*). $\times$ 9.6.

Fig. 2.—Vertical section through the upper part of the scolex corresponding to the line A-B in Fig. 1. The short parallel lines in the crests and bothridia indicate the disposition of the muscle fibres. $\times$ 31.

Fig. 3.—Similar vertical section, corresponding to the line C-D in Fig. 1. $\times$ 31.

Fig. 4.—Cirrus sac, cirrus and vagina in a much-flattened mature proglottid. The dilated fully-extended cirrus is seen to be inserted a considerable distance up the vagina, and the cirrus sac is very much contracted. $\times$ 14.

Fig. 5.—Part of transverse section through the subcuticula of a young mature proglottid to show the layer of longitudinal muscle fibres and the positions of the vitellaria and testes. $\times$ 144.

Fig. 6.—General anatomy of a ripe proglottid from the ventral aspect. The wide long uterine aperture (*UTO*) is conspicuous. $\times$ 14.

Figs. 7-10.—Transverse sections through a ripe proglottid. Fig. 7 is through the anterior end; Fig. 8 in the region of the anterior end of the uterine aperture; Fig. 9 through the cirrus sac and distal extremity of the vagina; Fig. 10 through the genital atrium. $\times$ 31.

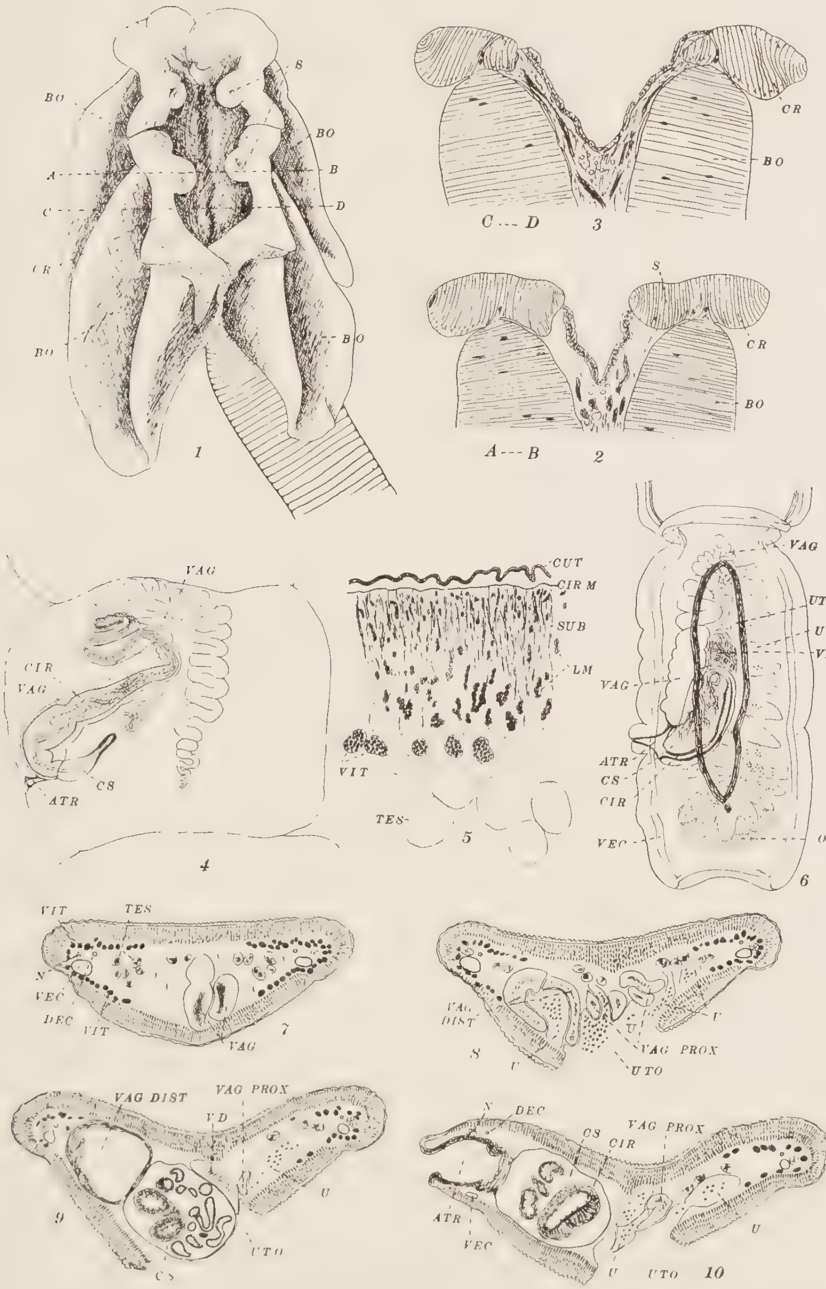
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PLATE XII

Explanation of Plate XIII

Figs. 11-13.—Transverse sections through a ripe proglottid. Fig. 11 through the anterior end of the ovary; Fig. 12 through the ovarian isthmus (the vagina is bending ventrally to open into the oviduct), and Fig. 13 through the posterior end of the proglottid and showing the excretory canals opening into the terminal bay. × 31.

Fig. 14.—Transverse section through a young mature proglottid in the region of the ovarian isthmus. × 31.

Parabothrium bulbiferum Nybelin.

Fig. 15.—The 70 mm. immature worm. ×0.8.

Fig. 16.—Scolex of the same magnified. × 2.

Fig. 17.—The scolex with the outer loose cuticula removed. × 2.

Fig. 18.—Diagram of longitudinal section through the scolex, showing the circum-medullary longitudinal musculature of the proglottids spreading thru the substance of the scolex. × 4.

Fig. 19.—Sketch of surface view of proglottis of a young mature worm. × 9.6.

Fig. 20.—Transverse section through a mature proglottid just anterior to the ovary to show the position of the testes, vitellaria, excretory vessels, nerves and the longitudinal muscle bundles. × 22.

Fig. 21.—The medullary zone of a typical proglottid in transverse section to show the number and positions of the excretory vessels. × 22.

Fig. 22.—The same to show the distribution of the vitellaria. × 22.

Fig. 23.—The cirrus sac and vagina in a composite (i. e., drawn from several actual sections) transverse section. × 70.

Fig. 24.—Transverse section through the region of the downgrowth (towards the exterior ventrally) from the uterus. × 70.

Fig. 25.—Sketch of the dorsal view (in a flattened preparation) of the main outlines of the ducts lying immediately anterior to the ovary. The vitelline ducts and shell-gland could not be distinguished. × 144.

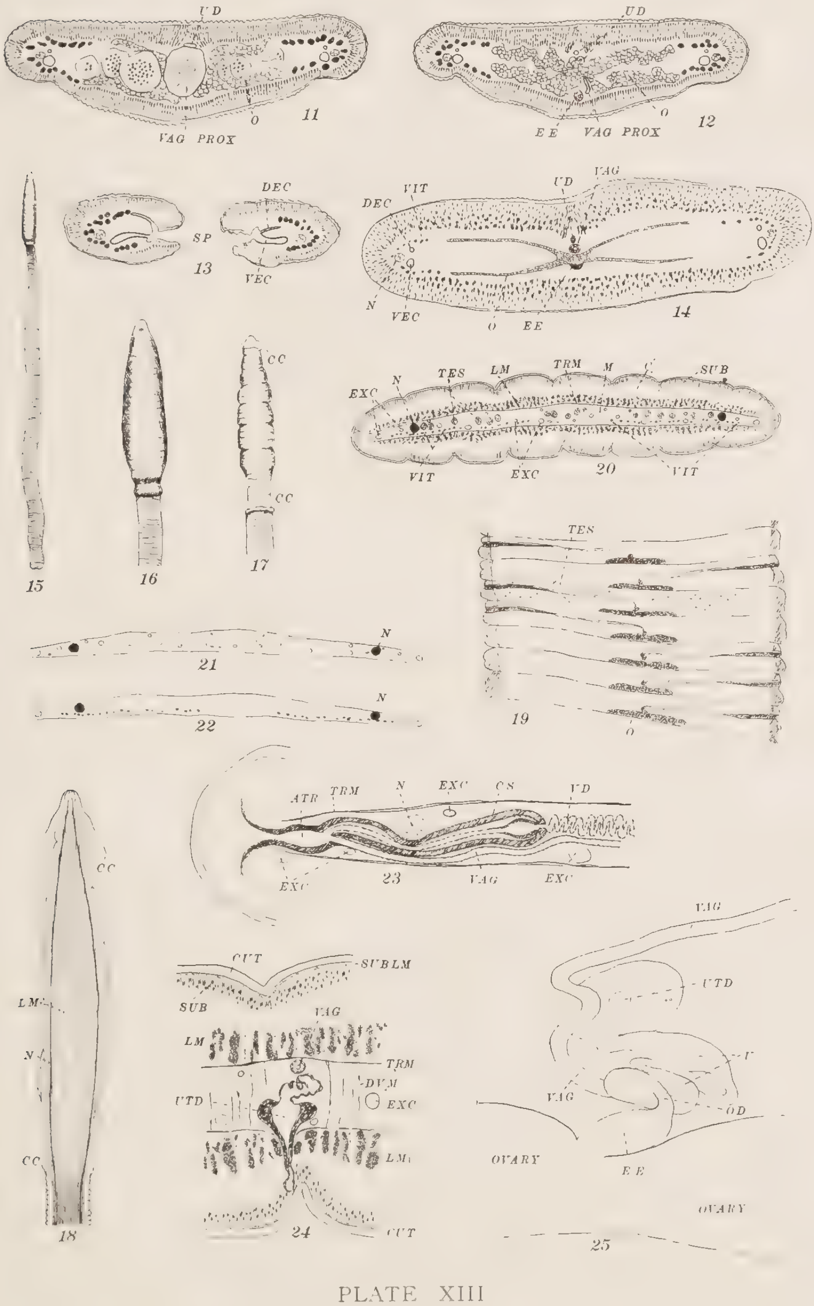
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PLATE XIII

STUDIES ON THE SOUTH AMERICAN TICK, *ORNITHODOROS VENEZUELENSIS* BRUMPT, IN COLOMBIA

ITS PREVALENCE, DISTRIBUTION, AND IMPORTANCE AS AN INTERMEDIATE HOST OF RELAPSING FEVER

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The following article on the South American relapsing fever tick, *Ornithodoros venezuelensis* Brumpt, embodies the results of investigations made during the period between July, 1923, and July, 1924, while the writer was engaged as entomologist and supervising inspector for the campaign against yellow fever then being carried on in Colombia. In September, 1923, Dr. Henry Hanson, director of the campaign, while examining some yellow fever suspects at Bucaramanga, discovered the spirochetes of relapsing fever in the blood stream of several of these individuals. Since it was known that *O. venezuelensis* was the transmitting agent of relapsing fever in the neighboring republics of Panama and Venezuela, the discovery of the presence of this fever in Colombia caused the writer to decide to take advantage of the opportunity to conduct some studies on this tick and its importance in the Republic. It was already known that this parasite was quite prevalent in some parts of Colombia, as the writer had found it to be numerous in houses in the town of Ebejico, which he had visited a few days before his arrival at Bucaramanga.

A number of specimens were obtained from four houses in Ebejico on August 26, 1923, and kept alive for later examination. Sixty-seven, fifty-five adults and twelve nymphs, were collected in one small abode hut. Many holes made by nails and tacks used in fastening up numerous small pictures, etc., were present in the whitewashed walls of the one room of this hut, and nearly every one of these holes was filled with the ticks. They were also found behind the pictures on the walls and in a wooden platform on the floor, which, when covered with a straw mat, served for the bed. Hundreds of ticks were present and many were filled with blood. There were three children in this hut and numerout small lesions caused by the bites of the tick were in evidence on their arms and legs. At the time these specimens were collected at Ebejico, it was not known to the writer that relapsing fever was of frequent occurrence in Colombia.

After discovering relapsing fever to be present in Bucaramanga a search was also made in that town for the ticks. They were found to be common in many of the houses and a number of specimens were

collected. One lot (No. 8) was obtained from a house where a child, three years of age, was sick with relapsing fever. The ticks were collected at night and were taken from holes in the wall, from the bed, and while crawling about on the walls. Another lot (No. 7) was taken from a house where a man was sick. Blood examinations of this man showed malarial parasites but no spirochetes. The ticks were not found to be numerous in this house but the marks on the walls where they had been killed well illustrated the efforts that were being made to keep the numbers reduced. The whitewashed walls of the small living room were practically covered with blood smears to a height of about three feet from the floor. The favorite position of rest of many people in this section, especially those of the poorer classes, is to sit on the floor with their backs against the wall, regardless of the number of chairs that might be present. This house, like many others in the town, had no floor except the ground. The ticks apparently took advantage of circumstances and probably many of them lived in the earth floor close to the wall thus making it very easy for them to crawl up on the people sitting against the wall. The blood smears were made by the people crushing the ticks while they were crawling about on the wall in search of a blood meal, or after having obtained one. Six other lots of ticks were collected in Bucaramanga and nearby towns. Those taken from each house were given a separate lot number for later examination.

Since the only means of transportation to or from Bucaramanga was by horse- or mule-back over a rough trail, a trip of several days, it was necessary to pack the ticks in secure containers, so that the rough handling given to baggage enroute would not break the containers and allow the ticks to escape. In view of this fact each lot was placed in a small cardboard pill box and the latter placed in tin tubes, of the kind in which kodak films are frequently packed for tropical use. The covers of the tubes were then sealed on with strips of adhesive tape. The ticks were left in these containers until March, 1924, when the writer, while enroute to the west coast of Colombia, via the Panama Canal, remained a few days at Ancon, Canal Zone, to test them for relapsing fever.

It was found that 256, or 72.72 per cent, of the ticks had died during the period elapsing between the time they were collected and their arrival at Panama. This high mortality was probably due to confinement in the pill boxes inside the tin tubes for so long a time. The ticks of four of the lots were all dead. This left ninety-six, or 27.27 per cent, to be examined.

Through the courtesy of the officials of the Health Department of the Canal Zone, this work was carried out at the Board of Health Laboratory at Ancon, C. Z. In making these tests each lot of ticks was macerated in 0.5 cc. normal saline solution and then injected, part intraperitoneally and part subcutaneously, into a white mouse or rat. The

animal used depended upon the number of ticks in the lot, mice generally being used for the small lots and rats for the larger ones. The blood of the animal was then examined daily during a period of fourteen days except when positive results were obtained earlier.

Of the nine lots tested, two gave positive results. Both of these had been collected at Bucaramanga. One lot (No. 7) was from the house in which the man was sick with malaria. It is possible that he also had had relapsing fever but the spirochetes were so scanty in the blood that they escaped detection at the time of the examination. The second lot (No. 8) had been collected in the house where the child had been sick with diagnosed relapsing fever. Both of these lots of ticks had been collected 172 days previous to being injected into the rats, and during this period they had been confined in the pill boxes and had not been fed. Had the tests been carried out sooner after the ticks were collected, or if there had been more of them alive to test, it is probable that more lots would have given positive results.

In April, 1924, an inspection trip along the west coast of Colombia afforded an opportunity for further observations on this tick and for collecting more specimens at the Pacific ports of Buenaventura and Tumaco, and at Barbacoas on the Telembi River in southwestern Colombia. At Buenaventura this parasite was extremely prevalent, being found in many of the houses in practically the same numbers that bedbugs are present in badly infested houses. Fifty-one ticks collected in one house were nearly all found in the joints and cracks of a small wooden stool used by the people for a seat, and from two door posts. The latter were simply small logs of soft wood about five inches in diameter. The bark had been removed and as the wood dried out many small cracks appeared in the posts which the ticks evidently found to be good hiding places. Fifty-four were taken from a bed in another small hut. Split bamboo was used for the floor of the bed and the ticks were numerous in the crevices present in this material. A grass mat found in a small, one-room hut, occupied by a negro dock laborer, yielded sixty-nine specimens. These were secured by spreading a bed sheet on the ground and then striking the mat on the sheet. This dislodged the ticks from the mat and they were then easily collected while crawling about on the white sheet. This mat was spread on the earth floor of the hut and used as a bed by the occupant of the hut. He stated that although he knew he received many bites each night they did not bother him much, and there were but few lesions that might have been caused by the bites in evidence on his body.

At Tumaco they were reported as being numerous in nearly all the houses and observations made bore out these statements. The ticks appeared to be present in greater numbers in the beds made of split bamboo than in the others. Probably the main reason for this is that the

many cracks made in the bamboo by splitting it open and flattening it out, provide innumerable places favorable for concealment and deposition of eggs. By holding the slabs of infested bamboo over a bed sheet or paper spread out on the floor and then striking it with a hammer or heavy piece of wood, a considerable number could be collected very quickly. More than one hundred were obtained from one bed by this method in a few minutes. At another house twenty-three were found in an old mosquito net and some rags arranged on one of the beds. At Barbacoas ticks were also present in large numbers. Specimens were secured in seven of the houses in a short time.

While traveling from Buenaventura to the Magdalena River via the Quinddio trail more collections were made at towns in the Cauca Valley and other places enroute. At Palmira, thirty-four specimens were taken in one house, all being found in a bed in which a sick baby, about one month old, was lying. The marks of a number of bites were in evidence on this baby. The prisoners in the jail at Palmira collected five specimens for me, but these were found only after a considerable search. I did not succeed in finding any at this jail during an hour's search that I made there one evening. While at Palmira a boy brought me 280 specimens that he had collected in little more than an hour at El Carmen, a small village about three miles from Palmira. At Ibagué nineteen ticks were taken from one house. Probably many more could have been secured at this town if further search had been made. During a brief search at Giradot on the Magdalena River thirty-one were found in a bed. They were very common at this town. At Barranquilla this tick was found to be quite prevalent in some sections of the city. Collections taken from nine houses totaled 1,168 specimens; 282 came from one house, more than 100 being found in the joints and cracks of several chairs and an old table. Specimens were also obtained from Soledad, a small village near Barranquilla. Puerto Colombia, the seaport for Barranquilla, was also found to be infested.

In June, 1924, while making a mosquito survey of the towns and villages on the Atrato and San Juan Rivers in the Choco District in the northwestern part of Colombia, additional collections were obtained from that region. At Quibdo, on the Atrato River, ticks were found to be extremely numerous and specimens were collected from five houses and the jail. At one house 106 were found in the crevices of an old, home-made trunk and seventy more were taken from the bed, chairs, and walls, making a total of 176. At another house some rough boards used for the floor of a bed had been placed on the ground outside just previous to our visit with the belief that the strong sunshine would kill the ticks in the crevices. A total of 120 were taken from the boards. One hundred and sixty-five were collected from the wooden bunks in the jail at Quibdo. Apparently some of the prisoners frequently

searched their bunks and made attempts to eliminate these pests while others made no such efforts. Collections were also made at Yuto and Lloro on the upper Atrato and at La Vuelta on the Andagueda River. At Istmina on the San Juan River this tick is very abundant and specimens were secured at seven houses. According to information given by some of the people a large percentage of the houses at Istmina were infested. Small collections were also received from Medina, east of Bogota, and from Muzo, northeast of Bogota. At Muzo, which has an altitude of about 2,700 feet, they were taken in the barracks of the guards at the emerald mines.

All the ticks collected at these various places were securely packed in containers and left until June, 1924, when they were taken to the Canal Zone and tested for relapsing fever as the previous lots from

TABLE 1.—*Data on Collections of Ticks*

Where Collections Were Obtained	Number of Ticks Col-lected	Number of Lots Repre-sented	Number of Lots Tested	Number of Ticks Tested	Results of Tests	
					Lots Positive	Lots Negative
Ebejico	109	4	3	16	..	3
Bucaramanga	233	8	6	80	2	4
Giron	10	1	0	0	..	..
Barbacoas	648	7	7	165	..	7
Tumaco	268	7	7	179	1	6
Buenaventura	263	5	5	141	1	4
Palmira	51	2	2	35	..	2
El Carmen	280	2	0	0	..	..
Ibague	19	1	1	6	..	1
Giradot	31	1	1	26	..	1
Soledad	4	1	1	4	..	1
Barranquilla	1168	9	9	471	6	3
Quibdo	638	6	6	542	2	4
Yuto	56	1	1	42	..	1
Lloro	90	1	1	72	1	..
La Vuelta	35	1	1	17	..	1
Istmina	850	7	7	644	4	3
Medina	27	1	1	26	..	1
Puerto Colombia	76	2	2	17	..	2
Muzo	24	1	0	0	..	..
Totals	4880	68	61	2483	17	44

Bucaramanga and Ebejico had been. Of the total number of 4,528 collected, 2,387 were alive to be tested upon arrival at the Canal Zone. All in the two lots from El Carmen and Muzo were dead. Those that remained alive in the other fifty-two lots were tested and positive results obtained in fifteen, representing 28.84 per cent. Combining these results with those previously examined gives a total of seventeen positives in the sixty-one lots tested, indicating that ticks infected with relapsing fever had been present in 27.86 per cent of the houses in which collections had been made.

It may be noted in Table No. 1 that the mortality among the ticks was quite high and that less than 51 per cent of the number collected were tested. Usually this tick is extremely long lived and this high mortality was probably partly due to being so closely confined in pill boxes for a considerable period of time and also in part to being injured during

collection. The inhabitants of many of the houses where search was made very often wished to assist in collecting the specimens and usually punctured many of them with pins or slivers of wood used for removing them from crevices in the walls or furniture. When the women or boys of a house were engaged to secure specimens they were very apt to obtain them by boiling water, gasoline, kerosene, etc., to drive the ticks out of their hiding places. All the specimens from Muzo, Giron, and El Carmen were dead by the time they reached the Canal Zone.

Several lots contained too many ticks to be injected into one rat or mouse and it was then necessary to divide them into sublots and use an animal for testing each sublot. This aided to some extent in determining

Table 2.—*Data on Positive Lots*

Place of Collection	Lot No.	Date Collected	Date Tested	Sublot No.	Number of Ticks	Animal Used	Result of Test	Interval Between Collecting and Testing
Bucaramanga	7	9/14/23	3/ 5/24	.....	6	Rat	Positive	172 days
Bucaramanga	8	9/14/23	3/ 5/24	8–A	12	Rat	Positive	172 days
Bucaramanga	8	9/14/23	3/ 5/24	8–B	12	Rat	Positive	172 days
Tumaco	26	4/ 5/24	6/21/24	26–A	7	Mouse	Negative	76 days
Tumaco	26	4/ 5/24	7/12/24	26–B	13	Rat	Positive	98 days
Buenaventura	29	4/ 7/24	6/21/24	29–A	15	Rat	Positive	74 days
Buenaventura	29	4/ 7/24	6/21/24	29–B	15	Rat	Negative	74 days
Barranquilla	40	5/22/24	7/10/24	.....	16	Mouse	Positive	48 days
Barranquilla	43	5/22/24	7/11/24	.....	174	Rat	Positive	49 days
Barranquilla	44	5/23/24	7/11/24	.....	17	Mouse	Positive	48 days
Barranquilla	46	5/23/24	7/11/24	.....	36	Mouse	Positive	48 days
Barranquilla	47	3/22/24	7/12/24	.....	10	Mouse	Positive	50 days
Barranquilla	48	5/22/24	7/12/24	.....	18	Rat	Positive	50 days
Quibdo	51	6/ 1/24	7/ 9/24	.....	125	Rat	Positive	38 days
Quibdo	53	6/ 1/24	7/ 9/24	53–A	74	Rat	Positive	38 days
Quibdo	53	6/ 1/24	7/ 9/24	53–B	70	Rat	Positive	38 days
Lloro	55	6/ 3/24	7/ 7/24	.....	72	Rat	Positive	34 days
Istmina	57	6/ 8/24	7/ 7/24	.....	63	Rat	Positive	29 days
Istmina	61	6/ 8/24	7/ 2/24	61–A	25	Mouse	Negative	24 days
Istmina	61	6/ 8/24	7/ 2/24	61–B	25	Mouse	Positive	24 days
Istmina	61	6/ 8/24	7/ 2/24	61–C	25	Mouse	Negative	24 days
Istmina	62	6/ 8/24	7/ 2/24	62–A	36	Rat	Positive	24 days
Istmina	62	6/ 8/24	7/ 2/24	62–B	30	Rat	Positive	24 days
Istmina	63	6/ 8/24	7/ 1/24	63–A	48	Rat	Positive	23 days
Istmina	63	6/ 8/24	7/ 1/24	63–B	40	Rat	Positive	23 days
Istmina	63	6/ 8/24	7/ 1/24	63–C	40	Rat	Positive	23 days

the infection of the lots, i. e., if a lot of sixty ticks gave an infection when injected into an animal it only proved that there had been at least one infected tick in the lot, although many more of them might also have been infected; but when the same number was divided into three sublots of twenty ticks each and three animals used, each positive result indicated that there had been at least one infected tick in each sublot of twenty.

The ticks of Lot No. 53 were collected in the jail at Quidbo. There were about thirty prisoners in the jail at the time and it is quite probable that cases of relapsing fever frequently occurred there.

These investigations show that a fairly high percentage of the *Ornithodoros venezuelensis* found in the various parts of Colombia are infected with the spirochete of relapsing fever and in view of this it is reasonable to believe that cases of this fever in man are much more

prevalent throughout the Republic than is commonly realized. The Choco region in western Colombia is apparently badly infested with this tick and travelers, especially foreigners who are likely to be non-immune to relapsing fever, while passing through that section, need to exercise precautions in selecting the places in which to spend their nights in order to avoid becoming infected. The *Boletin de Estadistica,* an official publication of the Department of the *Valle del Cauca,* for 1922, records thirty-seven cases of relapsing fever, nineteen occurring in males and eighteen in females, in that Department during the year.

The prevalence of these ticks in the houses at the seaports on the west coast of Colombia leads one to wonder how many cases of relapsing fever may have been diagnosed as yellow fever in the days when the latter disease was common in the Pacific ports of the Republic. It is also possible that relapsing fever may frequently be mistaken for malaria, especially where the microscope is but seldom used in making diagnosis. This may also be a factor in the rapid and successful results abtained by using an arsenical preparation, such as neosalvarsan, in some cases believed to be malaria.

This tick has various local names in the different sections of Colombia. At Bucaramanga it is known as *cuesca;* at Giradot as *chinche del tierra* from being frequently found in the earth floors of the houses; in Honda as *turicata;* in various villages along the Magdalena River as *berrinche;* at Barranquilla, Cartago, and throughout the Choco region as *chirivico* and *chinche garrapata;* at Tumaco and Barbacas it is called *chinche criolla;* and at Buenaventura it is known as *petacon* and *chinche sin olor.*

SUMMARY

A total of 4,880 ticks, *Ornithodoros venezuelensis,* was collected in sixty-eight houses in twenty villages, towns, and cities in various parts of Colombia. Sixty-one of the lots, consisting of 2,483 ticks were tested for relapsing fever and positive results obtained in seventeen, indicating that ticks infested with spirochetes of relapsing fever were present in seventeen, or 27.86 per cent, of the houses in which collections were made. These seventeen houses were located in seven different villages, towns, and cities showing that infected ticks have a wide distribution in Colombia. Three lots gave positive results after being closely confined in pill boxes for a period of 172 days, demonstrating that the spirochete may remain infective in the tick for that length of time without having had a blood meal.

I wish to express my thanks to Dr. Henry Hanson for the interest he took in this investigation and for his aid in collecting the ticks, and to Colonel H. C. Fisher, Chief Health Officer of the Panama Canal, and Dr. L. B. Bates, Director of the Board of Health Laboratories at Ancon, C. Z., for their kindness in placing all facilities of the Ancon Laboratory at my disposal in order that this work might be carried out.

DEVELOPMENT IN PRENATAL INFESTATION OF *BELASCARIS**

Donald L. Augustine

Prenatal infestation with parasitic worms has received considerable attention ever since Fujinami and Nakamura (1911) found *Schistosoma japonicum* in the fetus of a dog suffering from schistosomiasis. Following this observation, Narabayashi (1914) proved experimentally that prenatal infestation with *S. japonicum* is possible. He caused pregnant dogs to become infested with cercariae of *S. japonicum* and found the parasites in five out of seven embryos. Later on, fourteen young specimens of *S. japonicum* were found in the placentas of two pregnant guinea-pigs he had experimentally infested. That intra-uterine infestation with *S. japonicum* may occur under natural conditions was also shown by Narabayashi from twenty-two examinations of feces of new-born infants, three of which contained schistosome ova. The mothers of these infants gave a history of having worked in rice fields during their pregnancy.

Prenatal infestations with nematodes is believed to occur frequently. Since the report of Howard (1917) in which the finding of hookworm ova in the feces of a negro infant fourteen days old was recorded, a number of similar cases have been noted both in man and in lower animals. Of these cases those pertaining to the present subject are particularly interesting.

Fülleborn (1922) succeded in experimentally producing prenatal infestation of new-born dogs by injecting under the skin of a pregnant bitch thousands of *Belascaris marginata* larvae obtained from the liver of a guinea-pig which had been fed embryonated ova of these worms two days before. The puppies were born thirteen days after this inoculation and in each one hundreds of Belascaris larvae were found. At birth they were found in the liver and lungs. Two days later they were found passing through the trachea and esophagus to the intestine. The larvae found in the lungs measured about 900μ in length. Six days after birth many of the intestinal forms had grown to 4 mm. in length and after eleven days to 20 to 30 mm. About three weeks after the birth of the young dogs mature specimens of this parasite were found in the intestine.

Shillinger and Cram (1922) administered embryonated ova of *Belascaris marginata* per os to a bitch in advanced pregnancy. Eight days after this feeding twelve, well-developed puppies were born. Eight,

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however, were dead at birth and the remaining four died the following day. Eight of the twelve puppies showed infestation with larval Belascaris, six having them in the liver only, one in the lungs only, and one in both liver and lungs. None were found in the enteric canal. All larvae found were between 900 and 950μ in length. The bitch died eight days after the puppies were born or sixteen days after the inoculation. Post mortem examination showed no worms in the enteric canal but encysted larvae were found in the heart and lungs. This work of Shillinger and Cram confirms Fülleborn's findings as to the possibility of prenatal infestation with *B. marginata* and establishes the fact that following ingestion of infective Belascaris ova by pregnant animals, the larvae in the course of their migration may traverse the placenta and enter the young in utero.

The present series of experiments was planned originally to determine approximately the number of worms becoming established in new born dogs and in the mother from a given inoculation of infective *B. marginata* ova when administered during pregnancy. The outline of the first experiment was to give a pregnant bitch, free from intestinal nematodes, a known number of embryonated ova at a time sufficiently long before birth of the puppies for the larvae to become established in their intestines, and to make a numerical count of the worms found in the bitch and puppies at birth. With this end in view, a bitch, about one and one-half years of age, was given approximately 250 embryonated ova of *B. marginata* per os 33 days after coition. Twenty-seven days after her inoculation five healthy puppies were born. These puppies were born either late in the evening of December 7 or early on the following morning for they appeared several hours old the morning of December 8. Three of the puppies were chloroformed in the afternoon of December 8, and examination showed no worms in the intestine nor in pressed sections of fresh tissue of the liver, lungs, spleen and kidneys.

The fourth dog was killed on the following morning. Post mortem examination showed only larval forms in the bronchi and these were from 1 to 1.2 mm. in length. The fifth dog was killed when six days old, and thirty-three days after the inoculation of the mother dog. No worms were found in the lungs, liver, kidneys or spleen, but three immature worms 7 mm. long were found in the stomach and six more ranging from 3 to 6.2 mm. in length in the duodenum. This dog had been fed on pasteurized cow's milk for four days previous to this examination. The bitch was killed by intraperitoneal injection of chloretone two days after parturition. Post mortem examination showed no larvae in the liver, lungs, kidneys or spleen, but two young specimens 15.3 mm. in length were found in the duodenum.

A second bitch, about three years old, was similarly inoculated with a large number of embryonated ova of *B. marginata* on March 1, or 29

days after coition. On April 3, 62 days after coition and 33 days after the inoculation with the ascarid ova, nine apparently healthy puppies were born. The first puppy born was taken immediately at parturition and prepared for autopsy. In the liver, larval ascarids measuring between 0.7 and 0.9 mm. in length were found. No worms were found in the lungs, trachea, kidneys or intestine. A second dog was chloroformed and autopsied the following day or 24 hours after birth. Larvae 1 mm. in length were found in the lungs. All other tissues examined were negative. A third dog was killed April 5, or 48 hours after birth. At autopsy this dog showed lung larvae of 1 mm. in length. Dog number 4 was killed on the following day and likewise showed infestation only in the lungs. These larvae were, however, somewhat larger than those earlier encountered, and measured from 1.5 to 2 mm. in length. No infestation was found in puppies examined on the fourth and fifth days after birth. The seventh puppy was killed and examined April 9, or six days after birth. One ascarid, 4.3 mm. in length was found in the stomach and twenty-three, varying from 2 to 4 mm. in length, were taken from the jejunum. No infestation was found in the liver, lungs, or esophagus. The last two pups of this litter were fed on pasteurized cow's milk after April 10. On the 24th of the month one of the puppies appeared sick, showing marked weakness. It was killed on this day, 21 days after birth, and upon post mortem examination 53 young adult ascarids were found in the jejunum and duodenum. The worms were sexually differentiated although the females did not have ova in the uteri. The males averaged 16 mm. and the females 26 mm. in length. No infestation was found in the liver or lungs.

Fecal examinations by the Willis-Malloy salt flotation method were made daily on the last puppy. Ova of *Belascaris marginata* first appeared in the stools May 3, one month after birth or 64 days after inoculation of the mother dog. This pup was chloroformed and examined three days later. Twenty-eight male and 32 female ascarids were found in the upper part of the small intestine.

The bitch was killed by intraperitoneal injection of chloretone seven days after parturition. Upon autopsy no infestation was found in the liver, lungs or intestine. Here, as indicated in my first experiment, a decided preference is shown on the part of the Belascaris larvae for the fetal tissues. It is believed that all the worms found were present as a result of the feeding of the infective ova. The female dogs were obtained while in estrum and were kept throughout the experiment on asphalt floors which had been and were frequently scrubbed with boiling water. Both animals were found to have a light infestation with *B. marginata* upon their arrival at the laboratory and were given at this time 1 cc. of oil of chenopodium in a hard gelatin capsule followed by castor oil. A second treatment of the same drug was given one week later, although

it is doubtful if this was necessary as no stools were found positive for nematodes after the first treatment.

The experimental data of Fülleborn, Shillinger and Cram and this paper make a series of inoculations with embryonated *B. marginata* ova given to pregnant dogs 8, 13, 27, and 33 days before parturition. In none of the new born dogs were intestinal forms of this parasite found, and those encountered in the liver and lungs were all at the same stage of development, i. e., about 1 mm. in length. In the author's experiment where the pup was examined immediately after birth, larvae were found only in the liver. From one to three days later, they were found in the lungs and in dogs six days old the infestation was found located only in the stomach and small intestine. It, therefore, appears that the development of *B. marginata* in prenatal infestation is retarded in the fetus until parturition, after which migration takes place from the liver and lungs to the intestine, where the adult stage is reached.

The results of these experiments also indicate an age immunity in the bitch against this parasite. In the first animal inoculated with the embryonated ova a few worms were found in the intestine. The second one, at least a year older, received a great many more infective ova but showed no worms at autopsy. As *B. marginata* is considered a parasite of young dogs it is probable that the age of the pregnant dog may be a factor in determining the number of worms becoming established in the new-born litter.

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THE SEGREGATION OF LAMBS AT BIRTH AND THE FEEDING OF COW'S MILK IN THE ELIMINATION OF PARASITES *

Theobald Smith and E. Raymond Ring

The experiments to be described had their origin in the question of the significance of colostrum to the new-born lamb. The placenta of the cow is evidently impervious to antibodies and the colostrum takes over the transmission of these bodies to the new-born calf by way of the digestive tract. The similar structure of the ovine placenta naturally suggests a like function for the first milk. Experiments to test the protective value of colostrum were begun in 1924 by substituting cow's milk and the early results were such as to show clearly that the ewe's colostrum was not necessary to protect the lamb against miscellaneous infections and that normal growth took place even when no colostrum was fed. No further inquiries have been made in this direction, since bacterial diseases have not prevailed in the sheep making up the experimental flock. Measuring the accumulation of antibodies in the quiescent udder following active treatment of ewes with living infectious organisms and the tracing of such antibodies into the blood of the new-born lamb ingesting the colostrum are problems awaiting a suitable infectious agent.

The freedom from early diseases in spite of the feeding of cow's milk is to be assigned to the absence of infectious agents in the flock and the general indifference of sheep to bacterial diseases such as those which afflict calves. The result of withholding colostrum and feeding cow's milk should be watched with interest in flocks in which some bacterial disease-producing agent is enzootic. For the time being the indifference of lambs to withholding colostrum was utilized in a practical direction to determine whether under prevailing conditions and without too much personal attention lambs could be reared and maintained free from the numerous digestive and pulmonary parasites with which sheep are quite generally infested.

In 1922 and 1923, a group of 24 ewes and a ram were brought together from three different flocks. There were mixtures of Shropshire, Southdown, Hampshire and Cheviot breeds. During the spring of 1923, 27 lambs were born, and of these, 10 were left in the flock. During 1924, 37 lambs were born. Of these, 8 were segregated and included in experiments to be described. The remainder were left with the flock for a time. This was considerably reduced by the end

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of the year. In 1925, 24 lambs were born. Of these, 6 were secured for experimental purposes and 16 were left with the ewes. The entire original flock with contacts was finally disposed of in May, 1925. Fecal examinations and autopsies on individuals of this group from 1923 to 1926 showed the presence of the following parasites: coccidia, lung worms, stomach worms, Proteracrum (Oesophagostomum), Bunostomum, Trichuris, Nematodirus, and Moniezia.

FIRST EXPERIMENT

In 1924 the first attempts were made to segregate the new-born At lambing time, ewes were placed in individual stalls and were watched closely for signs of parturition. The stalls were kept clean and dry. Only lambs that were taken from their dams as they were being born were used in the experiments. Lambs born in the absence of an attendant were not used, even though they were discovered a few minutes later.

The lamb at birth was wrapped in a large towel and taken to a warm unit. There it was rubbed and dried carefully. One-half hour after birth warmed cow's milk was fed to the lamb. The milk was from cows that had been in lactation several months. It was fed from an ordinary 12 ounce nursing bottle through an ordinary rubber nipple after enlarging the hole. Bottles and nipples were scalded before each feeding. Some lambs took the first feeding without any difficulty, others had to be coaxed. After the first or second feeding no difficulty was experienced in getting the lamb to drink.

The amount of milk fed and the number of daily feedings depended on the size and condition of the lamb. As normal cow's milk contains more water, less protein and less fat than normal ewe's milk, it was deemed advisable to give as large an amount of cow's milk as the lambs could digest. Even then they did not seem to get enough nourishment. Too much milk, however, tended to distend the stomach abnormally. After a week, fresh water, hay, and a mixture of grain were kept before them. The grain mixture consisted of oats, cracked corn, bran, middlings, and a little bone meal. After a few days lambs began to nibble hay and grain. They were also given mangels and cabbage cut into small bits and grass in season. A piece of salt was kept in their grain dish. Lambs were docked when from 10 days to 2 weeks old and males castrated a few days later. As soon as there was plenty of green food in the fields, the lambs were weaned and put out of doors. Before this they had been taught to drink their milk from a pan so that the bottle and nipple could be discarded. When out of doors during the warm season the lambs were kept in a special enclosure in which was a small house for shelter. Care was taken to select land not hitherto passed over by sheep or else ploughed and

seeded to some crop during the preceding season. The enclosure was moved from time to time to fresh ground.

During the early months of 1924, four lambs (225, 229, 231, 245) were fed with the first milk or colostrum drawn from the ewe's udder and then cow's milk from a bottle. One (254) received only cow's milk from the start. Three (226, 228, 230) were allowed to suckle their dam: The udder was washed with soap and warm water and dried. The lambs suckled for about 10 minutes. They were then kept in a separate unit. This operation was continued for about 2 months until the lambs were weaned. After this they were given cow's milk in a trough. In all, 32 lambs were born during the season from February to April 3 and of these 8 were in the feeding experiment. It may be stated at the outset that this first attempt was not successful in eliminating parasites. The results obtained are however instructive and therefore very briefly given.

The lambs remaining with the ewes in the ordinary way were soon infested with the parasites of the preceding generation. Three lambs were examined, postmortem, one nearly 6 months, one slightly over 8 months, and one 10½ months old.

No. 253. Born March 18, died September 12. Very heavy infestation of fourth stomach with *H. contortus.* About 30 tapeworms in the small intestine. Nodules due to Oesophagostomum scattered along walls of ileum and large intestine with adult worms in latter situation. No macroscopic changes in lungs.

No. 240. Born February 25, killed November 5. In this animal *H. contortus* was in the fourth stomach. Worm nodules 2 to 10 mm. in diameter studded the walls of the small and the large intestine. There were few in the upper half of the small intestine. They increased in number downwards and were most numerous in cecum and upper colon. In jejunum groups of hemorrhagic spots 2 to 3 mm. in diameter. Two female Bunostomum in contents.

No. 238. Born February 24. Killed Jan. 7, 1925. In the caudal tip of both caudal lobes of the lungs are scattering, reddish, partly translucent areas, lying over firm, embedded foci ranging from mere points to 5 mm. in diameter. Terminal air tubes free from parasites. Sections show compact foci of lymphocytes, and foci of epithelioid cells enveloped in lymphocytes. Larger foci centrally necrotic. Air tubes markedly dilated. These lesions were due to lung worms, but none were seen in the sections examined. In the small intestine, five groups of petechiae in the mucosa. Worm tubercles appear in the lower half and are numerous in the walls of cecum and half way down the colon. One *H. contortus* found in fourth stomach.

In contrast to these are the results of autopsies on three experimental lambs, killed when 8⅓, 8⅓, and 10½ months old, respectively.

No. 228. Born February 18. Killed October 27. Allowed to suckle dam as described. Not in contact with older sheep. Autopsy negative. No worm lesions detected.

No. 245. One of triplets born February 25. Fed ewe's milk twice. Afterward cow's milk. Killed November 5. Weight, 95 pounds. Some tapeworms in the small intestine. Although *H. contortus* was not seen in fourth stomach, a few ova were found in the feces.

No. 230. Born February 19. Allowed to suckle the dam as described. No other contact with adults. Killed Jan. 7, 1925. Neither parasites nor lesions referable to them found at autopsy.

The examinations in these cases were not thorough but they were sufficiently demonstrative in view of the complete absence of worm nodules and lung lesions. In the control lambs kept with the ewes, the parasites were well established and abundant by the end of the first summer. The intestines were studded with many worm nodules. Lesions due to lung worms were in evidence. In the experimental lambs killed at parallel intervals the various parasites, although present in the group, failed to produce any recognizable lesions during the first year.

Subsequent examinations of the feces of the experimental group were made according to the concentration method devised by Clayton Lane (1923) and with his apparatus. The large amount of woody fiber and undigested vegetable fragments in animal feces made it necessary to place steel wool plugs in the tubes. This not only held down the coarser fragments but reduced the number of air bubbles on the cover slips topping the centrifuge tubes. Comparative tests with and without the steel wool plugs showed that ova were not strained out or held back by them.

The experimental lambs, although treated differently at the start, one group being placed on the ewe's udder at intervals, the others fed with the bottle, were brought together in a field enclosure May 9, as it was considered inexpedient to keep them in smaller groups. Hence distinctions cannot be drawn between the subgroups, nor can we trace the source of the different parasites. Since they were kept wholly segregated from older sheep and on enclosures on which sheep had not been kept, the parasites must have been transferred at birth or soon after when the lambs were fed from the ewe's udder.

One lamb was born in this group in 1925. No. 282, ram, born April 7, was allowed to suckle the dam and was kept continuously with this group. On Sept. 23, the feces contained many ova of *H. contortus* and about one-fifth as many of the lung worm ova, a few coccidia, and a very rare specimen of Bunostomum ova. The ram was killed December 18. The autopsy was negative with exception of a few rather firm, flattish nodules, 2 mm. in diameter, in the mucosa of upper half of small intestine. These did not contain any parasites. Worms were not detected by ordinary inspection of contents of digestive tract. However, fecal balls subjected to concentration contained a moderate number of *H. contortus* and lung worm ova. The results of examinations of feces (Clayton-Lane method) are given in Table 1.

It will be noted that coccidia are most widely distributed. Lung worms, *H. contortus*, and Bunostomum are present in the flock but not in all individuals and in very small numbers.

EXPERIMENT OF 1925

Twenty-four lambs were born in the original group of sheep during the lambing season of 1925. Of these, 6 were obtained at birth in a manner satisfactory for the experiment. Two were taken from the 1924 experimental group. These 8 were segregated and fed only on cow's milk as already described, with one exception to be given. They were fed 5 times daily for 2 weeks, and 4 times daily for about 3 months when milk was stopped. The quantity fed began with 2 ounces, or 60 cc. and rose slightly by ounces until at the end a pound, or about 500 cc. was fed at a single meal. The group was placed in an outdoor enclosure May 5. Care was taken as heretofore to select a pasture on which neither sheep nor manure from sheep had been placed, or else which had been ploughed and seeded in the preceding season. During 1925 the group was moved five times.

In May, 1926, they were moved to a partly swampy field through which runs a small brook. This field had been occupied by the original

TABLE 1.—*Ova of Parasites in Feces of Lambs of First Experiment (1924)*

No. of Lamb	Date of Birth	Dates of Examination		
		September, 1924	December, 1925	April, 1926
225	Feb. 7	H. contortus; coccidia	H. contortus; Bunostomum	H. contortus; one ovum (Trichuris ?)
226	Feb. 9	Lung worms; coccidia	H. contortus	H. contortus
229	Feb. 19	—*	0†	H. contortus
231	Feb. 19	—	0	H. contortus
		(June)	(September)	
282 (Lamb of No. 229)	April 7, 1925	0	H. contortus; lung worms; coccidia; Bunostomum	—

* — signifies that no examination was made.
† 0 signifies that ova or other indications of parasites were absent.

infested flock during the summer of 1923. In the fall the old flock was removed and one-half of the pasture ploughed and seeded to grass. In the spring of 1924 the same flock was returned to this pasture and kept there until late in November. In December, 1925, the dead grass was burned off but nothing more than this was done. In May, 1926, after the pasture had been unoccupied for 17 months, the lambs of 1925 were put in to determine whether the ova of the parasites left by the original flock had been destroyed during this period of one summer and two winters. The feces were examined after concentration by the Clayton-Lane procedure in May before they were placed in outdoor enclosures and again in September. In 1926, they were examined in April, July, and September. The July feces contained large numbers of a cestode ovum, which later was recognized as the ovum of *Moniezia expansa.* In an examination of mixed feces of this group made Nov. 18, 1926, a few coccidia were present and a single specimen of an ovum also detected in the feces of No. 272 in July.

This ovum was dark brownish, and measured 47μ by 33μ in both instances. The shell was thick (2.5μ) and radially marked with fine, closely set lines. The cytoplasm was contracted into a spherical, granular mass, coccidia-like, filling one-half of the shell and situated against one pole. The rest of the space within the shell was brownish and homogeneous. The characters of this ovum agree rather closely with those of a coccidium recently described by Spiegl (1925) which he names *Eimeria intricata.* He gives dimensions as 42 to 50.4μ by 30.6 to 36μ. Since only two individuals were observed in an entire preparation after concentration it was impossible to follow sporulation.

A synopsis of fecal examinations of this group is given in Table 2.

TABLE 2.—*Ova of Parasites in Feces of Lambs of Second Experiment (1925)*

No. of Lamb	Date of Birth	Dates of Examination					
		May, 1925	September, 1925	April, 1926	July, 1926	September, 1926	December, 1926
265	Feb. 1	0	0	Coccidia	Tapeworm ova	Coccidia	A few coccidia
266*	Feb. 1	0	0	0	Tapeworm ova; coccidia	Coccidia	One coccidium
271†	Feb. 7	—	0	0	—	—	—
272	Feb. 7	—	—	0	Tapeworm ova; one ovum, see text; coccidia	Coccidia	Three coccidia
273	Feb. 8	—	—	0	Tapeworm ova; coccidia	Coccidia	0
283	April 26	—	—	0	Tapeworm ova; coccidia	Coccidia	0
284	May 9	—	Coccidia	0	Tapeworm ova; coccidia	Coccidia	One coccidium (?)
268‡	Feb. 3	0	0	H. contortus; Bunostomum, one ovum	—	—	—

* Received at start 4 ounces of ewe's colostrum from bottle.
† Killed May, 1926. Prolapse and eversion of uterus. No worm lesions.
‡ Ram placed with 1924 group, October, 1925.

EXPERIMENT OF 1926

During the months of March, April, and May, there were born lambs in both the 1924 and the 1925 group. From the earlier group of ewes, 7 lambs were secured at birth, removed at once to a separate building, and reared on cow's milk. Four lambs were secured from the 1925 group and treated in the same way. Of the 11 lambs, some died or were killed early and they therefore did not enter the major experiment.

No. 286. Born March 8, one of triplets, was temporarily lame in left foreleg and fed cod liver oil for a time. It improved and had apparently fully recovered when the lambs were put in an outdoor enclosure May 17. It was found dead next day. The lungs were intensely congested and the liver quite fatty. In the thymus there were large hemorrhages. In sections of the liver were numerous microscopic hemorrhages. No other noteworthy changes found.

No. 288. Born March 8, also one of triplets, was deformed in its forelegs. It was weak from the start and died when 4 days old.

No. 296. Born May 16. The uterus of dam No. 271 became prolapsed and everted during parturition and she had to be killed. The lamb was lame in the right foreleg since birth. Although normal in other respects its joint did not improve. It was therefore killed when 2⅓ months old.

The remaining 8 lambs continued well and made the usual gains. They were kept stabled until May 17, then placed on a fenced square of land not known to have pastured sheep, with a small house as a shelter. The group was transferred to a fresh plot in August. This had been seeded to mixed hay in 1924. The feces of this group were examined three times during 1926 with results given in Table 3.

It will be noted that coccidia were quite uniformly present but at all times in small numbers. Two distinct types were observed, mostly together in the same sample of feces. One, rather small, with dimensions 23 to 28μ by 15 to 18μ; the other, larger, 35 to 46μ by 20 to 24μ. The latter had a polar lunule. Ova were found as follows:

No. 289. November 19. Two ova (60 by 28μ and 51 by 33μ), with double-contoured shell filled with granular material which in one ovum is imperfectly segmented into large balls (8 cell stage?).

TABLE 3.—*Ova of Parasites in Feces of Lambs of Third Experiment (1926)*

No. of Lamb	Date of Birth	Dates of Examination		
		June, 1926	September, 1926	November, 1926
287	March 8	—	Coccidia	0
289	March 14	—	0	Coccidia, 2 ova
290*	March 16	Coccidia, 1 ovum	—	1 tapeworm
291	March 16	—	Coccidia	Coccidia, 1 ovum
292	March 29	Coccidia, 1 ovum	Coccidia	0
293	April 13	Coccidia	Coccidia	Coccidia
294	April 26	—	Coccidia	Coccidia
295	April 29	—	—	Coccidia
296†	May 16	0	—	—

* Killed November 15 (see text).
† Killed July 26.

No. 290. June 29. One ovum 67μ by 28μ. The undifferentiated granular cytoplasm fills the shell.

No. 291. November 19. One ovum (65μ by 26μ), thin-shelled, filled with granular material.

These ova are nearly alike in size, not typical of those to be looked for as coming from the older flock. They are very scarce in the concentrated feces and may be ova belonging to other hosts which passed through the digestive tract.

The feces of No. 290 were overlooked in September. Being a ram, the animal was killed Nov. 15 as of no further use. Feces taken before death contained in the concentrate numerous cestode ova, like those found in the 1925 group in July. The autopsy showed complete absence of lesions referable to intestinal or pulmonary parasites, with the exception of several slightly elevated, flattish nodules, 2 to 3 mm. in diameter, in the upper small intestine. Parasites were not traced in these flat elevations. There was however present one tapeworm

(*Moniezia expansa*) discharging ripe proglottids which gave rise to the ova seen in the feces before death. These with envelopes measured in the longest diameters, 81 by 63μ.

DISCUSSION

The experiments covering three consecutive years plainly indicate that under the conditions colostrum and ewe's milk may be dispensed with, without bringing about any noteworthy injury or deterioration of the offspring. As stated at the outset, the presence of one or more enzootic infectious diseases in any flock may demand the use of colostrum for the initial protection of the newborn. This condition, however, did not play any part in these experiments.

The growth of the lambs fed on cow's milk was slightly retarded during the early months as compared with the lambs left with their dams. When placed in the outdoor enclosures they rapidly overtook the control group. Since all infested sheep and the lambs with them were disposed of, owing to the danger of a transfer of parasites to the experimental groups, no data are at hand bearing upon the influence of the parasites on later growth, fertility, condition of the wool, and sporadic deaths due to stomach worms and lung worms. It should be stated that sheep infested with the common parasites and kept in small fenced pastures would suffer most owing to the intensity of soil impregnation with parasite ova and larvae.

Turning to the effects of the experimental feeding on parasitism we find that even in the imperfect preliminary experiments of 1924, in which some lambs were allowed to suckle their dams at intervals, the latter show a remarkable improvement over the control lambs. Although probably nearly all types of parasites of the dams had passed to the lambs, the ova in the feces were scarce and the autopsies showed no lesions referable to lung worms or intestinal, nodule-producing forms.

The second experiment proved more successful. Coccidia were present in all members of the group in the second year. In July of this year, ova of a tapeworm (*Moniezia expansa*) were found in the feces of every animal. In September these had disappeared. Two specimens of an ovum of unknown source were found, one in July, the other in November.

The third experiment (1926) proved to be in its results similar to the second. Coccidia were encountered at all examinations. A few ova of unknown parentage came to light and one specimen of *Moniezia* in a lamb, killed when 8 months old.

The appearance of tapeworms in the second group when the lambs were 12 to 16 months old, and in one lamb of the third group, presents a problem which requires special study. The life history of this tape-

worm has not been cleared up, and its appearance in the second and the third group is thus capable of several different hypothetical interpretations. It is of interest to note that in the lamb harboring the tapeworms there was no disturbance of the normal formation of fecal balls, *i.e.* no diarrhea. The proglottids are evidently ground up in this normal process and none seen in fecal accumulations. On the other hand, the ova appeared in the concentrated fecal suspensions in large numbers. No mention is made of this passage of ova in the various compilations on the parasites of cattle and sheep. It appears to be assumed that the proglottids and chains of proglottids are to be recognized in the fecal masses. This may be true in diarrheal conditions.

The presence of coccidia may be accounted for in several ways. They may have been transmitted at the time of birth in a sporulating condition from feces attached to the wool and kept warm and moist there. They may be aberrant forms coming from other animal hosts living on the pastures. Usually two forms were present, readily distinguishable by their size and the possession of a polar lunule by the larger form. They occurred in such small numbers in the concentrated feces that any attempt made at the time to identify them by stimulating spore formation would have been futile. A third form, mentioned above, may have been present in the second group (1925). So far as could be ascertained, the coccidia did not produce any intestinal disturbance. They evidently multiplied very feebly, if at all, and the inference is not entirely excluded that they may have come from other hosts, may have been swallowed with the food and discharged without multiplying.

The few unidentified ova may represent true ovine parasites or they may be ova from other host species taken in with the food and discharged again. The final interpretation cannot be made until they have been entirely eliminated as foreign or until they have multiplied as native to sheep. This may require one or more seasons of observation on the 1925 and 1926 groups.

The disappearance of ova on the unoccupied pasture during the 17 months of vacancy seems to be fairly certain from the history of the second group (1925). The unexpected appearance of *Moniezia expansa* on this field does not demonstrate its survival there, since a single specimen was obtained from the third group (1926) which had been pastured at a considerable distance from this field and from any water courses or swamp land. The unexpected disappearance of this infestation is as mysterious as its appearance. Barring the appearance of the cestode for which an explanation is lacking, it may be concluded that the heavily infested feces of the original group deposited in large quantities on this land were rendered harmless during this fallow period.

The elimination of the nodule-producing nematode Proteracrum (Oesophagostomum) seems to have been successful, since nodules of this specific type have been entirely absent in lambs from the three groups autopsied from time to time.

It may be said that the method described for eliminating parasites has still to run the gauntlet of sheep infested with parasites of the skin (scabies, etc.). It offers no protection against insects, such as the screw-worm, nor against the various tapeworms in the life cycle of which the dog is involved. This latter group should, however, present no unusual difficulties.

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THE EFFECT OF SEA-WATER ON THE DEVELOPMENT OF HOOKWORM OVA AND LARVAE (*NECATOR AMERICANUS*)*

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In the search for a substance to destroy hookworm eggs and larvae, sodium chloride was one of the earliest to receive the attention of scientists. The toxic effect of common salt on the development of hookworm larvae was noted by Perroncito as early as 1880. Blanchard (1885) ascribed the immunity to hookworm infection among miners in the rock salt mines in Austria to the lethal action of the salt solution upon larvae. Boycott (1911) in England found that the miners of the Levant mine, in contrast with other tin mines, were free from infestation with hookworm due, presumably, to the effect of the sea-water which filtered into the mine. The absence of hookworm disease in various mines in Germany, France, and Italy was attributed to the salt content in these mines. Manouvris (1905), of France, advocated the use of salt solution in the disinfection of mines, and his method was extensively employed in Italy. Cort (1918) in a series of experiments in connection with the hookworm campaign in the gold mines of California indicated that salt has a high value as a disinfectant.

On the other hand, Neubert (Peipper, 1911), from a study of the infestation among native troops in German East Africa, concluded that sea-water has little effect as a disinfectant against hookworm. Nicoll (1917) in Australia from laboratory experiments indicated that the value of common salt as a disinfectant has been overestimated and that salt solution of less than 6 per cent is not more effective than rain-water under the same conditions. Recently Maplestone (1925) as the result of laboratory experiments stated that sea-water and sea-sand per se have no effect in preventing the development of hookworm eggs and larvae.

FIELD SURVEY

In the course of the hookworm campaign in Panama, in 1921, we learned that the San Blas Indians—men, women, and children—had the time-honored and inviolate custom of defecating directly into the sea below low tide. These Indians for the most part live on an archipelago of coral islands along the Atlantic Coast of Panama, where

* The studies and observations on which this paper is based were conducted with the support and under the auspices of the International Health Board of the Rockefeller Foundation.

conditions of soil, climate, rainfall and shade are most favorable for the development of hookworm larvae to the infective stage. Except for the disposal of all wastes into the sea, the living conditions of these Indians differ in no material way from those of the Panamanians on the mainland, where infestation with hookworm is heavy.

The situation offered an excellent opportunity for the study of the effect of such a method of sanitation on hookworm disease. A survey, therefore, was made of three of the most accessible of the islands, on which some non-Indians had had residence for five years. The 631 inhabitants were examined for intestinal parasites by the Willis method. Of 593 Indians only 38 or 4.7 per cent, were found infested with hookworms and all but one per cent of this infection could most reasonably be accounted for by occasional exposure to infestation on plantations on the mainland. Of the non-Indians 25, or 65.8 per cent, had hookworms. Among the Indians living in the interior of Panama 82.3 per cent were infested, and of 581 inhabitants of Donoso, a non-Indian village on the Atlantic mainland, 89 per cent had hookworms, as determined only by smears.

LABORATORY FINDINGS [1]

With a view to explaining the results of the survey, various experiments were conducted to determine the effect of sea-water upon hookworm eggs and larvae. In these we made use of quantitative methods involving the isolation of larvae from cultures containing a known number of viable hookworm ova.

The sea-water and sea-sand used in these experiments were obtained from the Gulf of Mexico, and would, therefore, have the average salinity of the North Atlantic Ocean. The sea-sand was taken when submerged under sea-water, and placed in air tight containers to prevent concentration of the salt content through evaporation. The control soil was sandy loam, previously found to give high yields of infective hookworm larvae. The sand obtained in the vicinity of the laboratory was a fine sand, resembling in texture that of the sea-sand. With the exception of the sea-sand, all soils were heated to destroy free living nematodes (Cort et al, 1922).

The feces were obtained from several persons. The majority of the cultures, however, were made from different specimens from the same individual. Ova of *Necator americanus* only were used. The proportion of feces to soils was constantly maintained in the ratio of 1 gram to 10. In order to make conditions uniform for comparison,

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1. This study was carried out at the Field Research Laboratory of the International Health Board of the Rockefeller Foundation, at Andalusia, Alabama. Because of the protracted illness of one of the writers, these experiments were not begun until October of 1925, postponed then because of unfavorable cultural conditions, and completed in the summer of 1926.

portions of the same specimen of feces were used throughout in any one series. The amount of feces present in a specimen determined, therefore, the number of cultures in any particular series and the amount of feces available for each culture, which varied from 2 to 10 grams; in the great majority of the cultures 5 grams were used. All specimens of feces were thoroughly mixed and the number of viable ova per gram determined by the method of the writers (Caldwell and Caldwell, 1926). The average of four counts from two portions was taken as the true count, disintegrated ova having been discounted.

The feces were mixed thoroughly with the soil, and sufficient liquid (tap water or sea-water) was added during mixing to make granular, flaky cultures, barely moist (Cort, 1925). Care was taken throughout to make all cultures of a uniform consistency. These were placed on filter papers, moist with sea-water or tap water as the experiment demanded, spread to a uniform depth of about half an inch in culture dishes, and incubated for 7 days, except as otherwise stated. During the period of the experiments artificial heat was not necessary to maintain optimum temperature conditions for the development of hookworm larvae. For the most part humidity was high, so that is was rarely necessary to moisten the cultures. Under these circumstances cultures were kept fairly constantly under like conditions, rendering comparison of yields of larvae more valuable. When artificial heat is necessary, with rapid or even moderate drying, the maintenance of equable moisture and temperature conditions is well nigh impossible.

Except in the dilution experiments the larvae were isolated in the modified Baermann apparatus (Cort et al, 1922). When the yield of larvae was very small, total counts were made. Otherwise the larvae were counted in two or three 0.15 cc samples of a suspension of larvae in water, the volume of which was adjusted to the yield of larvae, and from the average the number recovered per gram of feces was calculated. To ensure accuracy the larvae when active were killed by heat. From the experiences of others with culture and isolation methods (Stoll, 1923; Augustine and Smillie, 1926; Kerr and Rickard, 1926), confirmed in our series, it has become clear that comparison of results based upon such methods is valid only when sufficiently large numbers of cultures are run to rule out normal variations. The yields of larvae from the same specimen of feces vary with different soils, with changing conditions of aeration, temperature, and moisture in cultures. At best even under optimum circumstances, it is difficult to maintain absolutely uniform cultural conditions. Variations inherent in the methods were equalized (1) by repetition of experiments in series, and (2) by use of fecal specimens containing large numbers of viable hookworm ova, averaging 16,300 per gram.

Experiment 1 was devised to test the possible contamination of the shore with fecal material washed up by the sea. Five or ten grams of feces were placed (1) on sea-sand and submerged under sea-water, and, for comparison, (2) on sand submerged under tap water, for periods varying from 1 to 6 days. To avoid concentrating the salts in the sea-water, evaporation was made up daily by the addition of tap-water. For each series three control cultures were made to check the number of larvae recoverable under favorable conditions. At the end of each 24-hour period, the sea-water or tap water was pipetted off, the fecal material intimately mixed with the sea-sand or sand, the resulting saturated cultures incubated, and the larvae isolated and counted. (In this and the following experiment the drainage material was centrifuged, and the sediment isolated in the Baermann apparatus, to note whether larvae had hatched out and been withdrawn. Though records were kept of all such counts, the larvae were so few as to be negligible.)

TABLE 1.—*The Effect of Sea-Water Upon the Development of Hookworm Ova and Larvae. Submersion and Subsequent Culturing Under Conditions of Saturation*

Series	Viable Ova per Gram	Number of Each Cultured	Days Submerged	Average per Cent Recovered: Fresh Water	Average per Cent Recovered: Sea-Water
A	23,100	3	1	....	0.06
	Average controls 14,453 or 62.5%	3	2	....	0.03
		3	3	....	0.009
		3	4	....	0.009
B	8,700	2	1	6.7	0.002
	Average controls 7,720 or 88.7%	2	2	2.1	0.007
		2	3	4.0	0.003
		2	4	14.4	0.007
		2	5	19.9	0.05
C	14,200	1	1	3.1	0.004
	Average controls 8,533 or 60.9%	1	2	13.8	0.004
		1	3	14.0	0.000
		2	4	6.6	0.01

The results of this experiment (table 1) including comparison of 51 cultures, confirm the findings of others (Payne, 1922; Augustine, 1923; Maplestone, 1925), that the death rate of larvae under water-logged conditions of the soil is high, since the average number of larvae recovered from the water cultures is 9.4 per cent of the number of viable ova planted, in comparison with 79.8 per cent from the controls. They indicate further that under the same conditions, the lethal effect of saturation with sea-water is markedly greater, since the yield in such cultures averages but 0.015 per cent, or less than 1/600 that of the crop from the saturated water cultures.

In Experiment 2, to test the possibility of infestation by the transfer of fecal material washed up on the shore to more favorable conditions, Experiment 1 was modified as follows: After draining off the

sea-water or fresh water very carefully, the fecal material was transferred to, and mixed with, favorable control soil, and moistened with tap water. The results of isolations from these cultures, 152 in number in seven different series (table 2), suggest that submersion for one day under sea-water does not in all cases markedly prevent the development of hookworm ova to the infective larval stage. In two series the crops are comparable with the larval yields from feces submerged for

TABLE 2.—*The Effect Upon Hookworm Ova and Larvae of Submersion for Varying Periods in Fresh Water and Sea-Water with Subsequent Culturing Under Favorable Conditions*

Series	Viable Ova per Gram	Number of Each Cultured	Days Submerged	Average per Cent Recovered: Fresh Water	Average per Cent Recovered: Sea-Water
A	33,800	1	1	30.4	24.2
		1	2	33.2	0.06
		1	3	25.3	0.59
		1	4	15.4	0.00
B	6,600	2	1	43.7	37.0
		3	2	31.2	4.4
		3	3	37.7	6.3
		3	4	36.8	0.15
C	7,700	3	1	74.9	0.00
		3	2	55.6	0.00
		3	3	29.9	0.00
		3	4	42.9	0.00
		3	5	9.8	0.00
		3	6	11.1	0.00
D	16,300	2	1	76.7	26.5
	Average controls 12,284 or 75.3%	2	2	66.0	0.24
		2	3	58.9	0.16
		2	4	12.1	0.00
		2	5	3.5	0.00
E	17,500	2	1	71.5	0.00
	Average controls 16,433 or 93.6%	2	2	68.6	0.00
		2	3	50.1	0.03
		2	4	54.4	0.05
		2	5	10.9	0.00
F	10,600	2	1	69.0	12.6
	Average controls 7,377 or 69.6%	2	2	64.1	5.2
		2	3	47.4	1.0
		2	4	46.8	0.03
G*	33,400	2	1	54.4	2.9
	Average controls 20,503 or 61.3%	2	2	46.0	0.00
		2	3	28.6	0.00
		2	4	36.4	0.008
		1	5	9.2	0.00

* Cultures of last four days isolated after ten days incubation in order to determine whether a more prolonged period for development would give better crops of infective larvae in sea-water cultures.

one day under water, and in others the yield, though markedly less, is not negligible. The average recovery after one day's submersion is 14.7 per cent, with a range from 0 to 37 per cent. After two days' submersion, however, there is in all cultures a sharp drop in the number of larvae recovered from the sea-water cultures, approaching zero in the majority of cases. The average yield from all cultures, exclusive of the first day, is 0.75 per cent, with a range from 0 to 6.3 per cent. The average percentage of recovery from the water cultures

submerged for one day is comparable with the controls, averaging 60.1 per cent, with a range from 30.4 to 76.7 per cent. Thereafter, on the whole, there is a tendency to reduction in the crop with each day's submersion, becoming marked in some series on the fourth or fifth day. The average recovery for the second and third day is 46 per cent, with a range from 25.3 to 68.6 per cent; for subsequent days, the average yield is 24.1 per cent, with a range from 3.5 to 54.4 per cent. Whether or not the explanation for the slight recovery in the sea-water cultures under the conditions herein given lies wholly in the lethal effect of sea-water on hookworm *ova* during submersion will be discussed later.

Experiment 3 was designed to ascertain the effect of sea-water per se, i.e., without previous submersion. Series of cultures in control soil and sand were moistened (1) with tap water; (2) with sea-water.

TABLE 3.—*The Effect Upon the Development of Hookworm Ova and Larvae in Cultures Moistened with Tap Water or Sea-Water*

Series	Number of Each Cultured	Viable Ova per Gram	Average per Cent Recovered			
			Control Soil and Tap Water	Sand and Tap Water	Control or Sand and Sea-Water	Sea-Sand and Sea-Water
A	3	19,000	64.6	....	....	0.04
B	3	23,100	62.5	43.8	....	0.30
C	3	4,200	66.0	86.7	....	0.69
D	3	14,200	74.8	57.4	0.02	0.00
E*	6	13,200	82.1	62.3	C 0.01 S 0.00	0.02
F	3	5,800	60.3	79.5	0.32	0.00
G†	3	11,700	54.7	83.4	0.23	0.02

* Three each of series isolated in seven days; remainder in ten days.
† Isolated after 14 days to test whether development of ova was merely retarded by sea-water.

Cultures were also prepared in sea-sand by draining off the excess of sea-water with filter papers, leaving it just moist enough for good culture conditions. Some of the cultures in Series E were incubated for 10 days, and all of Series G for 14 days, in order to ascertain more certainly that sea-water had a lethal and not merely retarding effect upon the development of hookworm larvae to the infective stage. The results of this experiment, 80 cultures (Table 3), indicate clearly that both controls and sand when moistened with tap water under favorable conditions give high and comparable yields of larvae, with an average recovery, respectively, of 66.5 and 68.8 per cent of the number of viable ova planted. On the other hand, the yields from control soil or sand moistened with sea-water, but otherwise under identical conditions, give an average of only 0.116 per cent, or approximately 1/600 of the crop recovered from cultures moistened with tap water. The cultures of sea-sand are directly comparable with sand moistened with sea-water.

The remainder of the experiments reported in this paper were devised to ascertain the nature of the lethal action of sea-water on hookworm ova and larvae. In Experiment 4 cultures, seven each of the kinds compared, similar to those of the preceding experiment, were prepared, but instead of incubating for seven days prior to isolation, one each of the various cultures was isolated daily. For the sake of brevity, the tabular results of this and subsequent experiments are in large part omitted from this paper,[2] only sufficient being given for illustration. Experiment 4 includes the comparison of 266 cultures in 10 different series.

Considering first the results obtained with control and sand cultures moistened with tap water, the larval crops recovered the first two days seem erratic. They vary as between sand and control and in different series, in some markedly. The average recovery from control soils the first day is 25.9 per cent, with a range from 7.7 to 79.5 per cent in different series; from sand, the average recovery is 18 per cent with a range from 3.4 to 45.9 per cent. On the second day, the average crop from controls is 60 per cent, with a range from 42.8 to 78.6; from sand the average is 34.4 per cent, with a range from 15.7 to 52.7 per cent. From the third day onward, however, the percentages of crops of larvae recovered are on the whole comparable and fairly consistent, the average recovery from the third through the seventh day being 72.84 per cent in control and 69.01 per cent in sand.

Note was made of the stages of development of larvae. In general it may be stated that in the first isolation only immature larvae were recovered. The second day's crop showed a mixture of immature and developing rhabditiform larvae. On the third day well developed larvae approaching maturity were recovered in the majority of cases. On the fourth day a mixture of almost mature and infective larvae was the rule, the number of infective larvae present varying in different series. Thereafter, the picture revealed consistently large well-granuled infective larvae.

The explanation of the seemingly erratic variation in crops of larvae the first two days becomes clearer in the next experiment. It may be pointed out, however, that one cause of difference may lie in counting very young larvae. Newly hatched larvae have a tendency to collect in clusters, at times 50 to 100, making accurate dilution counts impossible. However, because of the great numbers of larvae dealt with and the repetition of the experiment in different series, general tendencies become evident. In isolation the more developed larvae come down singly. In isolations from sea-water cultures the larvae were counted dead or alive, as it was not certain whether the immature larvae recovered had died in the cultures previous to, or during, isolation.

2. The complete figures may be had from the writers.

Subsequent observations would suggest not only that the former interpretation was correct, but that some larvae were revived by the addition of water and that some ova were stimulated to hatch during isolation.

From a scrutiny of the results (Table 4) it becomes apparent that on the first two days only is the yield of larvae from a few of the sea-water cultures at all comparable to the yields from fresh water cultures. The average recovery for the first two days from sand and

TABLE 4.—*The Comparative Effects of Fresh Water and Sea-Water Upon Hookworm Ova and Larvae as Determined by Daily Isolations of Cultures**

			Per Cent Recovered per Gram			
Day	Series	Viable Ova per Gram	Control Soil and Tap Water	Sand and Tap Water	Control or Sand and Sea-Water	Sea-Sand and Sea-Water
1	A	15,700	37.1	46.9	26.2	11.2
	B	26,500	11.7	14.3	....	0.015
	C	11,900	79.5	33.1	1.1	0.003
	D	7,800	7.7	3.4	4.8	0.66
	E	15,900	12.7	3.8	4.6	0.05
	F	10,800	17.6	15.1	1.6	0.18
	G	25,200	15.5	13.2	3.8	1.3
	H	31,600	....	4.1	....	0.04
	I	11,700	....	24.2	12.5	1.17
	J	18,400	....	28.8	0.3	0.4
3	A	15,700	51.9	45.1	0.06	0.26
	B	26,500	60.2	35.2	....	0.007
	C	11,900	52.8	53.7	0.02	0.00
	D	7,800	85.4	70.9	0.03	0.006
	E	15,900	72.2	62.4	0.2	0.17
	F	10,800	87.8	86.4	0.03	0.02
	G	25,200	61.3	58.4	2.9	1.8
	H	31,600	....	44.7	....	1.5
	I	11,700	....	34.7	1.85	0.15
	J	18,400	....	69.5	3.18	2.3
6	A	15,700	84.2	71.6	0.22	0.02
	B	26,500	66.4	63.7	....	0.007
	C	11,900	70.8	73.0	0.0	0.00
	D	7,800	82.0	82.8	0.03	0.00
	E	15,900	51.9	60.8	0.006	0.00
	F	10,800	95.3	95.7	0.05	0.05
	G	25,200	42.9	43.3	0.22	0.19
	H	31,600	....	67.5	....	0.01
	I	11,700	....	54.7	0.004	0.00
	J	18,400	....	68.8	0.0	0.09

* Data for 2d, 4th, 5th and 7th days omitted for sake of brevity. Complete figures may be had from authors on request.

sea-water is 4.66 per cent, and from sea-sand 1.31 per cent, with a range from 1.14 to 26.2 per cent in the former and from 0.003 to 11.2 per cent in the latter. From then on, though there are variations in series and at times erratic increases, the larval recovery is spectacularly and consistently low in comparison with yields from fresh water. The predominant picture in all sea-water recoveries, from the first through the seventh day, is that of newly hatched larvae—mostly dead from the third day on. In a few of the cultures from the fifth day some partially developed larvae were present among the generally prevailing immature specimens, and a few infective larvae were recovered, the greatest number being 10 in the culture isolated on the seventh day in Series G, in which had been planted 25,200 viable ova per gram.

The average recovery (including all larvae) from the third through the seventh day from sand and sea-water cultures is 0.75 per cent and from sea-sand 0.33 per cent. Although Experiment 4 suggested the nature of the lethal action of sea-water, it was not conclusive.

Experiment 5 was undertaken to make clear just what was happening in the cultures through differential dilution counts. Series of cultures were made as in the preceding experiment (omitting the sand

Table 5.—*Daily Differential Count Showing the Comparative Effects of Water and Sea-Water on Hookworm Larvae*

Day	Culture		Dead	Alive	Larvae Description	Embryo in Shell	T. P.	Mor.	Dis.
1	S & W	A	..	74	Immature	21	4	1	0
		B	..	44	Immature	56	0	0	0
		C	..	11	Immature	85	2	2	0
		D	..	37	Immature	62	0	1	0
		E	..	89	Some development	7	1	3	0
	S & SW	B	..	0	....	98	1	1	0
		C	..	0	....	96	1	2	1
		D	..	0	....	98	0	0	2
		E	..	14	Immature	82	2	1	1
	SS & SW	A	..	1	Immature	88	6	1	4
		B	..	0	....	93	3	1	3
		C	..	0	....	92	4	1	3
		D	..	0	....	99	0	0	1
2	S & W	A	..	99	Fairly developed	1	0	0	0
		B	..	98	Immature and partially developed	0	1	1	0
		C	..	92	Immature and partially developed	6	1	0	1
		D	..	89	Immature and partially developed	11	0	0	0
		E	..	94	Well developed, majority	5	0	1	0
	S & SW	B	..	18	Immature	78	1	0	3
		C	..	35	Immature	58	1	2	4
		D	..	79	Immature, slightly developed	19	1	1	0
		E	..	27	Immature	67	0	3	3
	SS & SW	A	..	6	Immature	87	6	1	0
		B	..	8	Immature	90	2	0	0
		C	..	4	Immature	87	5	4	0
		D	..	56	Immature	44	0	0	0
6	S & W	B	..	100	Healthy active infective larvae	0	0	0	0
		C	9	90	Healthy active, majority infective larvae	1	0	0	0
		D	..	100	Healthy active infective larvae	0	0	0	0
		E*	..	..	....	..	..	..	..
	S & SW	B	84	3	Immature	8	0	1	4
		C	89	0	Immature (motionless or very sluggish)	8	0	0	3
		D	37	63	Slightly developed to almost mature larvae.	0	0	0	0
		E	91	0	Immature	5	0	0	4
	SS & SW	B†	61	..	Immature	28	3	5	3
		C	79	..	Immature	16	0	0	5
		D	70	21	Slightly developed (sluggish)	2	1	0	6

* Control inadvertently destroyed.

† Larvae so disintegrated, impossible to get accurate differential count.

loam cultures). In three series (A, B, C) small cultures were made and one each examined daily. In two series (D, E) larger cultures containing 10 grams of feces were made and equal portions of these removed for daily examination. To these water was added, suspension was secured by bubbling through a pipette, and differential counts were made of the first 100 ova or larvae noted on microscopic examination of samples of this suspension. Now a clearer picture is obtained of the action of sea-water.

The results [3] (Table 5) indicate, first, that in sand and water cultures under equally favorable conditions of temperature and moisture, the proportion of larvae hatching during the first two days varies with different feces. In series in which counts were also made from control soil, development apparently was somewhat more rapid in sandy loam. These facts in a measure explain the variation in crops of larvae recovered from water cultures in different series in the preceding isolation experiments. The picture revealed in dilution differential counts is in general agreement with the results obtained in Experiment 4, i. e., varying proportions of larvae hatching out the first two days, with progressive development of the larvae to the infective stage. Infective larvae first appear in numbers on the fourth day and from then on the predominant picture is that of active, healthy, infective larvae.

The effects of sea-water apparently are (1) delay in the hatching of the embryos, (2) swift death to the immature larvae. Under the moisture conditions in these cultures, the sea-water clearly does not to any extent prevent the development of the ova to the embryo stage. In 24 hours the predominant picture is that of embryos ready to hatch, but none or very few larvae. During the next 24 hours varying proportions of larvae hatch out, 4 to 79 per cent, more in sand and sea-water than in sea-sand. From this day on, a few larvae hatch out daily, but they die promptly in the majority of cases without further development (the exception of Series D will be discussed later). On the whole, the larvae which hatch out one day are dead the next and show disintegration. On the second day of observation—the first in which larvae hatch out in any number in the sea-water cultures—the newly hatched larvae are fairly active; but in subsequent days those alive are sluggish; movement is almost imperceptible in the majority. Except for those just hatched, it is probable that the movement of others is due to the stimulus of the water added to make the dilution counts. The longer the ova remain in the culture before the embryos hatch, the less vigorous do the larvae appear. Some die in the act of hatching, too feeble to clear the shell; others clear the shell with difficulty and subsequent movements are very feeble, in high contrast to the almost instantaneous hatching of embryos in the water cultures and the active movements of the larvae. By the fifth day the majority have hatched and died. The counts of embryos in the shell do not distinguish between viable and non-viable embryos. From the fourth day onward, however, some of the unhatched embryos become glassy and there is evident shrinking. By the end of the week practically all larvae are dead, and the ova remaining, particularly those containing embryos, are no longer viable.

Looss (1911) in his monumental work suggests that the inner vitelline membrane, even more than the outer shell, serves as a protective

3. See note in connection with Experiment 4.

armament against the deleterious effects of various chemicals. As the embryos approach maturity, this membrane loses its protective power to enable the hatching embryos to escape. Our results are in accord with this explanation. The quantitative results revealed in the daily isolation and dilution counts are in general agreement with the earlier qualitative findings of Bruns (1904) and of Boycott (1911). Lambinet (1906) confirmed the finding of Bruns that a 3 per cent solution of salt is the minimum concentration for preventing the formation of (infective) larvae.

The North Atlantic sea-water contains 3.79 per cent total salts, of which 2.94 per cent is sodium chloride. The total salt content approaches closely to the concentration of salt solution found by Bruns to prevent the development of hookworm larvae. In making cultures by the addition of just sufficient sea-water to produce optimum moisture conditions for larval development, the actual salt content in any given series of cultures depends upon the character of the feces. In any culture the salt content of the resultant liquid medium surrounding the ova is less than in sea-water. With feces of high moisture content, the concentration of the salt may be reduced below the point sufficient for marked lethal effect upon the hatching larvae. This fact may serve to explain variations in crops of infective larvae recovered from sea-water cultures, and is a possible explanation of the partial development of larvae from sea-water cultures in Series D, as the feces used were very soft and but very few drops of sea-water were added to make cultures of the proper consistency. This is evident from the fact that in Series E, which contained unusually healthy young larvae in water cultures, they promptly died without developing in sea-water cultures

In connection with this discussion the results of Experiment 2 are of interest. These tended to show that submergence under sea-water for more than one day effectually prevents the development of hookworm ova to the infective stage. It must be pointed out, however, that the fecal material transferred was, on the whole saturated with sea-water, so that little additional tap water was necessary in creating optimum moisture conditions in culturing. The sea-water was drained from the "plants" so that they appeared dry, but the fecal material itself had absorbed sea-water. In all cases, of course, since the cultures were moistened with tap water in mixing, the resulting medium had a salt content less than that of sea-water. The results in Table 2 do not, therefore, necessarily indicate that submergence for more than one day render the ova nonviable. They may signify that larvae hatched out from ova submerged under sea-water for more than one day are unable to resist the lethal action of a moisture medium lower in salt content than sea-water.

Experiment 6 was carried out to test the full effect of sea-water upon young larvae. Because of the consistent findings of the lethal effect of sea-water—even diminished in salt content—in preceding experiments, this was designed to be suggestive only. Sand and water cultures of the same series as "E" of Experiment 5 were used as a basis. Differential counts of 100 from portions of five separate cultures showed such remarkable agreement as to suggest strongly that differential counts give a true cross-section of what is happening in the cultures as a whole. Counts from five sand and sea-water cultures served to emphasize this conclusion. In the sand and water cultures practically all ova had hatched (average 95 per cent) in 24 hours and the greater proportion of them showed some development. They represented, then, unusually vigorous larvae. One of these cultures was thoroughly saturated with water, and differential counts were made for four days. Despite this soaking with water, the results in general agree with those of the same series in Experiment 5 under favorable conditions of moisture. Though some died, 84 per cent active healthy infective larvae were present on the fourth day. An equally vigorous culture was at the same time saturated with sea-water to the same extent as the water culture. All larvae died and showed marked disintegration in the following two days. A very few of the remaining ova hatched out and the young larvae promptly succumbed.

SUMMARY

1. Examination of 593 San Blas Indians, who habitually defecate directly into the sea below low tide, showed only 4.7 per cent infestation with hookworm, in contrast with 82.3 per cent infestation among Indians in the Interior of Panama, and 89 per cent in a non-Indian village where soil pollution was the rule.

2. From quantitative experiments undertaken to explain the findings of the survey the following results were obtained:

(*a*) Under conditions of saturation sea-water has a marked lethal effect on the development of hookworm ova to the infective larval stage, 600 times that of fresh water under like conditions, the average recovery being 0.015 and 9.04 per cent respectively.

(*b*) Under optimum cultural conditions, the lethal action of sea-water was demonstrated by an average larval yield of 0.116 per cent in contrast with 67.6 per cent from cultures moistened with tap water.

(*c*) From feces containing viable hookworm ova submerged under sea-water for more than one day and transferred to favorable conditions of temperature, soil, and moisture, the larval yield was consistently low, averaging 0.75 per cent from all cultures. The lethal action of sea-water may in this case be due to its effects on (1) ova, and (2) young larvae, because of the residual salt content in the feces. Results were compared with fresh water cultures under like conditions.

3. Quantitative experiments involving daily isolations and differential dilution counts from cultures revealed that under optimum cultural conditions:

(*a*) Sea water does not to any extent prevent the development of hookworm ova to the embryo stage.

(*b*) It retards the hatching of the embryos.

(*c*) It kills the newly hatched larvae.

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SOCIETY PROCEEDINGS

AMERICAN SOCIETY OF PARASITOLOGISTS

The eighth regular meeting of the Council of the American Society of Parasitologists was held on April 16. The final report of the treasurer for 1926 was as follows:

Balance on hand, Jan. 1, 1926	$ 0.89
Dues from 349 members for 1926	349.00
Total receipts	$349.89
Expenses for year	374.46
Deficit	$ 24.57

It was duly audited by P. Bartsch and accepted by the Council.

The secretary reported that the present membership of the society was 424, of whom 307 resided in the United States and 117 in foreign countries.

The question of the advisability of printing abstracts of papers submitted at the Nashville meeting was discussed and final action left in the hands of the program committee. The Council commented favorably on the suggestion for a symposium on the problems of medical parasitology and for a discussion on the teaching of parasitology, together with an opportunity for demonstrations at the Nashville meeting. A program committee was elected for that meeting, consisting of W. W. Cort, chairman; R. W. Hegner, F. D. Barker, and H. W. Brown.

W. W. Cort, *Secy.-Treas.*

It is not too early to urge upon the attention of the members the importance of this meeting. The program committee will welcome suggestions and titles. While the Nashville meeting may not attract as large an attendance as has been brought together at some greater centers, the importance of animal parasites in the South will give this society and its program an unusually prominent position in the series of meetings held at Nashville during convocation week next December. It is the confident expectation of the officers that the American Society of Parasitologists will do full justice to this opportunity, but if such is to be the case, the officers must have effective support from the full membership.

CHINA BRANCH OF THE AMERICAN SOCIETY OF PARASITOLOGISTS

A meeting of the China Branch of the American Society of Parasitologists was held in the Peking Union Medical College Friday, January 28, at 4:30 p. m. The meeting was well attended. Dr. E. C. Faust, the chairman of the China Branch, presided. The secretary, Dr. C. F. Wu, reported an increase of sixteen members since the September meeting which brings the total number of members for the China Branch up to fifty.

The following program was presented:

Dr. J. F. Kessel spoke on the Intestinal Protozoa of Monkeys. Whether the protozoa of monkeys are the same species as the ones found in man is a problem of both theoretical and medical importance. Intestinal protozoa have been found in monkeys from Asia, Africa, and South America and for the most part there is agreement that they are all morphologically similar to the intestinal protozoa of man. Some investigators hold that physiological differences may exist between the two groups, a question which can be determined only by experimental work along the following lines. First, by cross-animal experimentation with the two

hosts in question, second, by comparison of their growth *in vitro* in the same culture media, and third, by the infection of a standardized animal, such as the kitten, with the pathogenic species. In the present investigation the intestinal fauna of twenty monkeys has been studied and protozoa morphologically similar to *Endamoeba dysenteriae, Endamoeba coli, Iodamoeba bütschlii, Endolimax nana, Giardia lamblia, Chilomastix mesnili, Trichomonas intestinalis* (both triflagellate and tetra-flagellate forms), and *Embadomonas intestinalis* have been encountered. All of these forms with the exception of Giardia have been cultured with facility in the same culture media in which the intestinal protozoa of man are easily cultivatible, while *E. dysenteriae, E. coli,* Iodamoeba, Endolimax, Chilomastix and Trichomonas from man have been experimentally transferred to the monkey. The dysentery amoeba of the monkey has been experimentally transferred to kittens both by means of cysts and by trophozoites from culture. Eight out of thirteen kittens have acquired the infection, six succumbing to amoebic dysentery similar to the type found in kittens infected with *E. dysenteriae* of man. Sections of the pathological lesions showed the same characteristic ulceration and penetration into the tissue as the writer has found in kittens infected with the human dysentery amoeba. The dysentery amoeba of monkeys has been reported in numerous instances to produce dysentery and liver abscess in monkeys. In this study amoebae have been found in the lymph glands and muscularis of the intestinal wall of the monkey, while the dysentery amoeba has been found to ingest red blood corpuscles of the monkey, of kittens and of man. Trichomonds of the monkey and of man have also been transferred to kittens.

A mensurative study of the trophozoites and cysts of Giardia of the monkey show them to be of very nearly the same size and proportions as Simon's figure for the trophozoites of Giardia of man and as Hegner's figures for the cysts of Giardia of monkeys. Six hundred Giardia cysts from ten human cases measured by the writer show a much closer size resemblance to the cysts of Giardia from monkeys than did Simon's, his mean ratio of length to breadth being 1.37 while the writer's is 1.61. Two hundred and fifty cysts of Chilomastix from man and the same number from monkeys show no appreciable difference in size or in ratio of length to breadth.

In view of the above findings it is concluded that at present there are neither morphological nor physiological differences between the intestinal protozoa of man and of monkeys which warrant the protozoa of monkeys being classified as separate species.

Dr. Henry E. Meleney spoke on The Types of Breeding Place Used by *Anopheles hyrcanus* in North and Central China. Observations were made during the summer and autumn of 1926. In general the larvae of this mosquito were found in water which was still and clear and which contained water plants of the genera Spirogyra and Ceratophyllum. Ponds up to 100 meters in diameter were by far the most frequent breeding places. Canals contained larvae only where they were little used and had the appropriate water plants. Occasionally larvae were found in slowly running streams, always in places where Spirogyra was present. Occasionally, in still water, larvae were found in the absence of the usual water plants. In this case fish were never present in the water. Fish are undoubtedly important in preventing the development of larvae of this species. In ponds in Nanking used for raising fish, where water plants were absent no larvae were found. Other ponds with abundant water plants of the genera mentioned were good breeding places. A study should be made of small fish native to China which may act efficiently as larva destroyers. Domestic water receptacles are not used by this species. Rice fields were occasionally found to contain larvae in small numbers. The decision as to whether they are important breeding places must await further observations. The measures which will probably be found to be most efficient against the breeding of this mosquito are:

1. Drainage and filling of ponds.
2. Keeping the surface of ponds free from water plants.
3. Stocking ponds with larvivorous fish.
4. Spraying ponds with paris green.

Dr. Chenfu F. Wu described two flatworms from the Soochow area, a trematode from the buccal cavity of the frog and a larval tapeworm from the mesentery of the water snake. The fluke belongs to the genus Halipegus, but differs in several respects from the described species of this genus. The larval cestodes belongs to the genus Ophiotaenia, and are characterized by the presence of a very well-developed fifth (anterior) sucker.

Dr. E. C. Faust reported on four species of linguatulids which had been found in man and other hosts in China. All of the specimens were nymphs. *Armillifer moniliformis* (Diesing 1835), peculiar to the Oriental region, had been obtained from a Tibetan who had died in Peking of miliary tuberculosis. The worm was encysted on the margin of the liver and was walled off from the liver tissue by a thick fibrous capsule. Its integument was provided with spines, which had been described for the nymphal stage of other genera of linguatulids but had not been recorded for this genus. This was the first record of the occurrence of this genus in China and the third human case. *Linguatula serrata* Froehlich 1789 had been found in the respiratory passages of a laboratory rabbit in Peking in 1921. All of the specimens examined had the characteristic two pairs of claws and, in addition, the immature claws of the next (adult) stage, a condition noted by Leuckart (1860), but not referred to by later investigators. This was the first record of the genus and species for China. *Kiricephalus pattoni* (Stephens 1908), which had been previously recóvered from a Hongkong snake (Southwell 1924), was reported from the lungs of a cat, autopsied in Changsha. This suggested that felines were the natural hosts of the larval stages and that they incurred their infection from consumption of the viscera of snakes. These three species belong to the subfamily Porocephalinae, characterized by the presence of two pairs of circumoral claws in the larval, nymphal and adult stages and by a posteriorly disposed utero-vaginal pore. The fourth species, *Reighardia sternae* (Diesing 1864), previously described only in the adult stage from the air sacs of terns and gulls, had been obtained from the portal blood and lungs of *Sterna fluviatilis*, captured on the shore near Tientsin. The worms were nymphs and were evidently in migration from the intestine to the air sacs. The adults of this species had been previously found to have two pairs of claws and an anteriorly disposed utero-vaginal pore; the unhatched larvae had been found to possess three pairs of claws. The nymphs in this collection were like the embryos in possessing three pairs of claws. This finding gives further support for the differentiation of this genus along with Raillietiella from the other described linguatulids and the erection of the subfamily Raillietiellinae Sambon 1922 for them. This is the first record of a species of this subfamily in the Far East. The study of these Chinese linguatulids supports Sambon's classification and nosogeographic data.

Dr. Wu Lien Teh spoke of the recent outbreak of pneumonic plague in Mongolia (fall of 1926), on the motor road between Urga, the principal trading center of Mongolia and the Manchurian border. Only a few cases were known to have occurred. Due to precautionary measures instituted by the Manchurian Plague Prevention Service in cooperation with local authorities the disease was kept out of Manchuria. The tarbagan is the reservoir host of the disease. Human infection is first contracted in the bubonic form, by hunters who catch the tarbagan for its fur, the disease being conveyed either by direct contact or by the bite of the tarbagan flea which serves as a vector. The hunters, in turn, transfer the infection in the pneumonic form to other individuals. Dr. Wu also referred to the helminth parasites of the tarbagan, stating that in a few cases an ascarid had been found which was very closely related to *Ascaris lumbricoides*.

Drs. C. W. Young and M. Hertig gave a demonstration of the periarticular lesions in hamsters infected with kala azar.

BOOK REVIEWS

Protozoology, a Manual for Medical Men, Veterinarians and Zoologists, by C. M. Wenyon. William Wood and Company, New York, 1926, 2 vols, 1,563 pp.

This magnificent work by the well known protozoologist constitutes not only in size but also in character easily the greatest contribution on this group which has appeared for many years. There is little doubt that it will remain for all time one of the significant manuals in the field. While written with the purpose of serving particularly the technical interests of certain professions, it deals with the subject in such broad fashion that it may properly be designated a comprehensive treatment of the entire subject of Protozoology. To be sure the parasitic species are most fully discussed and special emphasis is laid upon those forms which infect man and other animals most intimately related to him. Nevertheless, the relation of these forms to free-living species and to the intermediate groups of the coprozoic types are sufficiently fully discussed to unify the treatment of the subject. The entire work falls into six sections. The first, a general description of the protozoa covering 150 pages, takes up questions of structure, life history, activities, and special relations to drugs. At first this section appears unfortunately brief in relation to the later parts of the treatise, but after all it is somewhat a matter of judgment as to what might be found here and what might be given in later sections. Furthermore, it is evident that this section is only intended as an introduction and consequently has been held down to the minimum so that all possible space might be devoted to the following sections. This justification applies least of all to the portions on immunity and the action of drugs. The French investigators during researches especially on Trypanosomiasis have contributed findings concerning the production of resistant strains and other features both of marked biological interest and of outstanding importance also from the medical standpoint.

The second part is a discussion of the structure and classification of the coprozoic and parasitic protozoa and is encyclopedic in character, covering in all more than a thousand pages and embracing such a myriad of minor items, cross-references to other forms, and suggestions regarding the significance of certain details that the reader is forced to stop constantly in order to pay his respects to the author's command of the field. Among the major divisions included, one is struck by the presence of the Spirochaetes. Not only in earlier writings but here also Wenyon emphasizes the fact that in all probability these are not protozoa and then proceeds to discuss them, since as he says their consideration is so intimately associated with the study of protozoa by virtue of their occurrence that on practical grounds it seems necessary to include them in this work. The human parasites are described with great precision and in each case the author takes up structure, life history, experimental culture, pathological significance, symptomatology, diagnosis, and response to the action of drugs. With great fair-mindedness the author has included also as doubtful forms those which have been accorded an independent status by other authors, even though he himself has regarded the type in question as a variant of some other species. Suggestions as to the interpretation of these doubtful forms impress one frequently as most admirable; as, for instance, his acceptance of the view that the coccidium *Eimeria wenyoni* and related species, are in reality fish parasites which gained access to man through contaminated food and really never had even a temporary relation in more than mechanical fashion to the human body.

If one were to venture a criticism on the arrangement, it would be that the headings lack uniformity in style and consequently it is difficult to trace organisms either of the same systematic grade or similar in their relation to the human

host; and the method of presentation leads one at first to the erroneous assumption that among the parasites in man are certain forms which really are in no wise related to him. Such confusion is perhaps not strange in view of the fact that the author considers in some detail several hundred species and lists in the index several thousand.

As a well-trained zoologist Wenyon has handled the complex question of nomenclature with fairness and yet firmly. For the assistance particularly of physicians and veterinarians he has included the International Code of Zoological Nomenclature, a document which it is difficult for anyone to find in printed form and which unfortunately is almost unknown outside of limited groups among zoologists. The author has also given a splendid bibliography of this subject and abundant cross-references to it in the text. While careful checking has disclosed a few errors, they are indeed rare. The amount of first-hand information made accessible is, in the opinion of the reviewer, larger than has ever before been placed at the disposal of the workers in any similar treatise.

The appearance of the book is attractive; the figures are numerous, new, and very good. Special praise should be given to the twenty splendid colored plates with which the two volumes are supplied. Certainly the publishers have given to the work a setting of which it is most worthy and of which they may well be proud.

THE BIOLOGY OF THE PROTOZOA. By GARY N. CALKINS. Lea & Febiger, Philadelphia, 623 pp.

No one is better fitted by virtue of study and research to write a successful treatise on the single-celled organisms than the author of this recent volume. It is a mine of information well arranged, and attractively set forth. The method of treatment makes it particularly valuable for the general student in that he finds at once an immense amount of scattered information sorted and arranged under biological headings and in the form in which he can best use the material. However, even specialists are profited by the work Calkins has done in placing together in orderly form the large amount of detail on these forms that has been recorded by a multitude of authors within very recent times.

The various structural features are treated extensively and the vital processes form also subjects of individual chapters or sections. In view of the splendid treatment given other biological problems one must regret that the author has contented himself with statements regarding parasitism so brief that they are hardly more than passing comments. If the reader of the work could have found a section on that topic it would have been of interest as it is certainly of importance in view of the rôle played by the unicellular organisms in the causation of disease.

Several chapters are devoted to structure and one to the taxonomy of each of the major subdivisions; here the student of parasitology will be pleased to find mention of many parasitic groups and the best synopsis of Sporozoa available within the given limits. Each chapter has a separate bibliography and a general list of publications is to be found at the close of the book. Unfortunately these references are not always uniform. In one place, for instance, the same periodical is referred to in different fashion in successive lines and in both cases inaccurately. This and other errors in citation are not likely to be serious for the experienced worker but often occasion confusion and loss of time for those who are less familiar with literature.

THE CESTODES OF MAMMALS. By F. J. MEGGITT. Edward Goldston, London, 282 pp.

The work done by Professor Meggitt in preparing this book will be greatly appreciated by a multitude of students and workers in parasitology. He first prints a synopsis of the classification of Cestoda and follows that by brief diagnoses of orders, families, genera, and other major subdivisions of the group

with keys for each subdivision except the genera. The individual species are listed with author, date, and synonyms, and under each species the known hosts. Larval forms are handled as well as adult cestodes, and a list of hosts grouped taxonomically is followed by an index to Cestodes, a list of names omitted and a host index. The record of the literature closes a work that must have entailed on the author much labor. The work is well done and merits unstinted praise despite some unfortunate typographic errors that escaped the author's notice in correcting proof.

Syphilis, Paludisme, Amibiase. By Paul Ravaut. Masson et Cie., Paris, 1927. 284 pp.

The volume is devoted to methods of treating three maladies which rightly rank among the greatest curses of the human race. The three diseases agree in that the causal organisms are protozoa and also in that they display great sensitiveness to specific medication. The author's style is characteristically French in its clarity and while concise covers thoroughly the subjects mentioned. Professor Widal of Paris has written an interesting preface for the book.

In the second volume of *Collected Addresses and Laboratory Studies,* Dr. Leiper has brought together a goodly series of papers from the London School of Hygiene and Tropical Medicine, some thirty-five titles in all by twenty-four authors. The wide variety of subjects treated is adequate evidence of the breadth of the work and opportunities at the school.

Studies on Tsutsugamushi Disease. By Dr. Rinya Kawamura. Authorized English Translation. Edited by Drs. N. C. Foot and S. Tashiro. The Medical Bulletin, College of Medicine, University of Cincinnati, special numbers 1 and 2, Vol. IV; 229 pp., 50 textfigures, 25 plates.

The valuable monograph on Japanese flood fever which was published originally in Japanese and has been known to workers heretofore only in the form of abstracts, has been translated in full with the approval of the author, and now appears in English. The translation, which has been carefully edited and compared with the original Japanese edition by Doctors Foot and Tashiro, may undoubtedly be regarded as thoroughly accurate. The importance of the topic justifies one in stating that in making the translation and presenting it to the scientific world, both the translaters and the publishers have rendered a genuine service to scientific medicine and to parasitology. The work includes extensive and conclusive clinical observations on tsutsugamushi disease, further studies on the pathology of the malady, a record of experimental work on laboratory animals, a careful presentation of the etiology of the disease, a discussion of the mite responsible for its transmission, and finally a record of experiments and other data bearing on the methods for the prevention of the disease. The work is well illustrated and has a full bibliography, including references to papers by Japanese, American and European authors. Recent discoveries concerning tularemia in North America add especial interest to the appearance in English of Kawamura's monograph.

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